GRAIN HARDNESS AND SLOW DRY MATTER DISAPPEARANCE RATE IN BARLEY

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ABSTRACT

Barley grain is an important source of energy and protein for ruminant animals. However, feeding must be carefully managed to avoid maladies caused by the rapid breakdown of barley starch in the rumen. The development of slower degrading barley for ruminants may alleviate health problems associated with barley grain consumption. Selection for hard endosperm may result in slower starch degradation and improved feed quality. The objectives of this study were to: examine the effect of grain hardness, variety and environment on dry matter disappearance rate (DMDR); identify accurate and efficient hardness selection tools; and study environmental effects, inheritance and heritability of hardness.

To study grain hardness and genetic and environmental effects on DMDR, two genotypes grown at multiple locations in 2004 were analyzed for Single Kernel Characterization System (SKCS) hardness, by scanning electron microscopy (SEM), and for *in situ* DMDR. Genotype by environment interaction influenced DMDR, while neither SKCS hardness nor SEM analysis accurately differentiated DMDR between genotypes.

Eight genotypes were grown at multiple locations during 2003 and 2004 to study grain hardness measurement methodology, and genetic and environmental effects on hardness. Genotypes were analyzed for SKCS hardness, milling energy, endosperm light reflectance, feed particle size, protein and beta-glucan. Hardness measurements ranked genotypes similarly across environments. Feed particle size was correlated with milling energy but not other hardness measurements. Hardness measurements appeared to be influenced by protein and beta-glucan.

To examine the inheritance and heritability of barley grain hardness, 245 double haploid (DH) genotypes and parents, grown in 2003 and 2004, were analyzed for SKCS hardness, milling energy, protein, beta-glucan, with 100 evaluated for light reflectance. The population exhibited normal distributions for SKCS hardness, milling energy, protein and beta-glucan, suggesting quantitative inheritance for these traits with no apparent epistatic gene interaction. Narrow-sense heritability was 0.75 for SKCS hardness and 0.41 for protein. Light reflectance was not normally distributed, suggesting complementary gene interaction. Broad-sense heritability was 0.53.

Barley grain hardness is highly heritable and an efficient tool in making selections in a breeding program. However, breeding for high beta-glucan and protein may be better selection criteria for indirect selection of DMDR.

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LIST OF ABBREVIATIONS

A – rapidly soluble fraction (*in situ* disappearance)

AAFC – Agriculture and Agri-Food Canada

B – potentially degradable fraction (*in situ* disappearance)

C – undegradable fraction (*in situ* disappearance)

CDC – Crop Development Centre

DH – double haploid

DM – dry matter

DMD – dry matter disappearance

DMDR – dry matter disappearance rate

EDDM – effective degradation of dry matter (*in situ* disappearance)

GD – Goodale Crop Research Farm, Saskatoon

KCRF – Kernen Crop Research Farm, Saskatoon

ME – Milling Energy

PSA – Particle Size Analysis

QTL – Quantitative Trait Loci

RCBD – random complete block design

RIL – recombinant inbred line

RUDM – rumen undegradable dry matter (*in situ* disappearance)

SEM – Scanning Electron Microscopy

SF – Seed Farm, Saskatoon

SKCS – Single Kernel Characterization System

1. INTRODUCTION

Barley is grown in a wide array of environments but is best suited to cool and dry temperate climates (Poehlman, 1985). Of the cereal crops, barley has the broadest adaptation and is generally grown in climates less suitable for other cereals.

Barley is the fourth largest cereal crop produced in the world. The versatile composition of barley makes it suitable for feed, malt and food. Worldwide, barley is predominantly utilized as feed (70%), with 20% used for malt, only 5% for food, and 5% for undefined uses (Wang, 2005a). In Canada, feed consumption of barley has increased from 60% to nearly 80% of total domestic barley use since 1991, due to strong growth of the Western Canadian livestock industry (Wang, 2005b). Barley starch is easily accessible to enzymatic breakdown, resulting in readily available energy for growth. However, careful management is required when feeding barley grain to ruminants. Rapid starch breakdown can lead to health problems, such as acidosis, parakeratosis, ruminitis, liver abscesses, and bloat (Orskov, 1986).

Grain hardness has been described as the resistance of the kernel to fracture (Anjum and Walker, 1991). In barley, grain hardness is gaining importance in quality determination, while the wheat industry has used it for decades to differentiate grain quality and market classes. Hard wheat kernels require more force to fracture while soft wheat grains require less energy, caused by differences in the endosperm starch-protein matrix. Grain hardness has also been described as the extent of endosperm packing (Holopainen et al., 2005). Mealiness describes loosely packed cells with air spaces between starch granules while steeliness describes densely packed cells forming a dense starch-protein matrix (Chandra et al., 1999).

Grain hardness appears to be heritable. In barley, grain hardness has been studied in various ways, including via the Single Kernel Characterization System (SKCS), Milling Energy (ME), Scanning Electron Microscopy (SEM), Light Transmission and Particle Size Analysis. Although initially developed for wheat, the SKCS has found application in other grains, including barley. In barley, Nielsen (2003) reported significant correlation between SKCS hardness and malt modification and friability, making it a good predictor of malt quality. In food

barley, Washington et al. (2001) found the SKCS a valuable tool in identifying barley pearling quality and uniformity for the Japanese food (rice extender, miso and shochu) market. They indicated that SKCS hardness coefficient of variation was the best predictor of pearling yield and broken kernels. In feed barley, Beecher et al. (2002) found a small but significant relationship between SKCS hardness and dry matter digestibility and proposed further research with application in feed quality selection.

Milling energy (ME) is predominantly applied to barley for malting. Allison et al. (1976) established that milling energy differentiates between good and poor malting barley varieties, with good malting varieties requiring less ME.

Scanning Electron Microscopy (SEM) of endosperm cells allows visual observation of the starch-protein matrix and compaction characteristics while Light Transmission collects similar information on whole kernels.

Particle Size Analysis is the measurement of particle size after grain milling or feed processing, and is a long-used method to evaluate end-use quality. It is important to note that milled flour particle size is measured at the micron level rather than the larger millimeter sieves used in feed analysis. Flour particle size can be used to identify hard or soft wheat, which determines end-use suitability (Morris and Rose, 1996). Soft wheat fractures easily into flour with small particle size and limited starch damage, while hard wheat produces larger flour particles with increased starch damage. Researchers have found that ruminant performance is significantly influenced by the particle size of the feed consumed. Galyean et al. (1981) reported a negative relationship between feed particle size and dry matter disappearance of maize and sorghum. Similar results for maize and barley were reported by McAllister et al. (1993) and for barley by Bowman et al. (1996).

Dry matter disappearance (DMD) can be described as the rate and/or extent of feed breakdown in the rumen (Orskov et al., 1969; Mehrez et al., 1977). Measuring DMD is critical for optimizing feeding practices, especially in cereal rich diets high in readily available energy. Feeding high concentrations of cereal grains to ruminants can lead to maladies including acidosis, bloat and liver abscesses (as reviewed by Galyean and Rivera, 2003). Dry matter disappearance of cereals is dependent on complex, interrelated factors, including feed composition, consumption over time, mechanical and chemical alterations of the feed, and adaptation of rumen microorganisms (Huntington, 1997). Cereal species differ in DMD and its

relation to starch digestion in the rumen. Orskov (1986) summarized several studies and concluded that at least 90% of barley and wheat starch is fermented in the rumen, compared to about 60% for maize. This slower digestion of maize is preferred to avoid health problems, such as acidosis, bloat and liver abscesses (Orskov, 1986). Variation in DMD also occurs between types within the same cereal species. Feed particle size influences DMD primarily because of differences in exposed surface area per unit volume with larger particles having comparatively less area exposed. As microbial attachment to particles is essential for rumen fermentation (McAllister et al., 1994), larger particles with less exposed area per volume degrade more slowly.

Conventional breeding of feed barley varieties for ruminants has relied on selection for physical grain characteristics such as bulk density, kernel weight and plumpness (Ullrich, 2002). Selection for larger feed particle size and lower acid detergent fiber (ADF) to improve feed barley for ruminants has been suggested by Bowman et al. (1996) and Surber et al. (1999). Surber et al. (2000) reported that extent of rumen DMD was inversely correlated to mean feed particle size ($r = -0.57$) and ADF content ($r = -0.81$).

During malting, soft barley modifies more easily as enzymes are more able to attack the starchy endosperm. Conversely, hard barley has incomplete or delayed endosperm modification. It is the hypothesis of this research that hard barley would degrade more slowly in the rumen due to more difficult microbial access to the barley starch in the rumen. The goal is to determine an efficient grain hardness evaluation technique that could be used to screen large numbers of lines in a breeding program for slow DMD rate. The objectives of this thesis research were to: examine the effect of grain hardness, variety and environment on dry matter disappearance rates; identify accurate and efficient grain hardness selection tools for plant breeding; and study environmental effects, inheritance and heritability of grain hardness.

2. LITERATURE REVIEW

2.1 Barley

Cultivated barley (*Hordeum vulgare* L.) belongs to the tribe Triticeae and the grass family Gramineae (Briggs, 1978a). It is a self-pollinating diploid plant with seven paired chromosomes (2*n*=14) (Briggs, 1978b). Barley is grown in a wide array of environments but is best suited to cool and dry temperate climates (Poehlman, 1985). Of the cereal crops, barley has the broadest adaptation and is generally grown in climates less suitable for other cereals.

2.1.1 Production

Barley is the fourth largest cereal crop produced in the world (FAO Statistics, 2006), ranking behind maize, wheat and rice, respectively. From 1996 to 2005, world barley production averaged 143 million metric tonnes (FAO Statistics, 2006), while in Canada, 12.5 million metric tonnes per year was produced (Statistics Canada, 2006). More than ninety percent of Canadian barley is grown in the Prairie Provinces of Alberta, Saskatchewan and Manitoba. In comparison, these provinces produce merely 3% of the 8.5 million metric tonnes of Canadian maize per year (Statistics Canada, 2006). Only wheat at 24.5 million metric tonnes per year surpassed barley in total Canadian cereal production (Statistics Canada, 2006).

2.1.2 Grain Composition

Barley kernels are normally comprised of 80% carbohydrate (dry weight), with the vast majority (65%) occurring as starch (MacGregor and Fincher, 1993). Similar to wheat and maize, normal barley starch is 75% amylopectin and 25% amylose. Like wheat, barley starch granules exist in two forms – large (15 to 25μm) lenticular A-type granules and small (<10μm) spherical B-type granules. Small B-type granules comprise 80 to 90% of the total starch granules by number but only 10 to 15% by weight. Of note, maize starch granules are found only in spherical form, ranging in size from 5 to 30 μm (Watson, 1987).

Cell wall associated polysaccharides represent the second largest carbohydrate fraction in barley, making up to 10% of total grain weight (MacGregor and Fincher, 1993). This fraction is primarily (1-3), (1-4) beta-D-glucan, originating mainly from endosperm cell walls, which are about 75% beta-glucan (Fincher, 1975). Total grain beta-glucan levels range from 2 to 11% (by weight) in barley with most averaging 4 to 7% (MacGregor and Fincher, 1993). In contrast, limited amounts of beta-glucan have been reported in wheat (0.52 to 1.0%) (Beresford and Stone, 1983) and maize (0.5 to 1.3%) (Demirbas, 2005).

Barley grain protein normally ranges from 10 to 16% (dry weight), compared to wheat (10 to 15%) and maize (7 to 13%) (Simmonds, 1978). Thirty-six to 49% of total barley protein is comprised of hordein storage protein and concentration is strongly influenced by soil nitrogen availability (Kirkman et al., 1982). Generally, barley protein contains lower levels of the essential amino acids lysine, methionine, tryptophan, and threonine than required for human and animal nutrition (Newman and Newman, 1992). Low lysine levels are due to the abundance of hordein, which normally contains less than 1% lysine, compared to 5 to 7% for the non-storage albumin and globulin proteins (Kirkman et al., 1982). Similar to wheat and maize, low lysine levels in barley render it nutritionally unbalanced for humans and non-ruminant livestock (Foster and Prentice, 1987).

Lipid concentration of barley generally ranges from 2 to 3% (Welch, 1978) with reports of cultivars as high as 5.3% (Bhatty and Rossnagel, 1980). Wheat lipid levels of 2 to 4% (Johnson and Mattern, 1987) are similar to barley. However, maize contains more, ranging from 3.5 to 6% (Glover and Mertz, 1987). Barley embryos are 18% lipid by weight (Bhatty and Rossnagel, 1980), compared to wheat and maize at about 30% and 35%, respectively (Johnson and Mattern, 1987; Earle et al., 1946).

2.1.3 Uses

The versatile composition of barley makes it suitable for feed, malt and food. Worldwide, barley is predominantly utilized as feed (70%), with 20% used for malt, only 5% for food, and 5% undefined uses (Wang, 2005a). In Canada, domestic demand for feed barley has increased from 60 to nearly 80% of total production since 1991, due to strong growth of the Western Canadian livestock industry (Wang, 2005b).

Where barley is an important source of feed energy and protein for ruminant and monogastric animals, a typical diet for finishing beef cattle may include >80% barley grain (Wang et al., 1999). Subject to price and availability of feed wheat, swine rations may include >70% barley grain (Patience et al., 1995).

Barley starch is easily accessible to enzymatic breakdown, resulting in readily available energy for growth. However, careful management is required when feeding barley grain to ruminants. Rapid starch breakdown can lead to health problems, such as acidosis, parakeratosis, ruminitis, liver abscesses, and bloat (Orskov, 1986). In poultry, starch accessibility is hampered by the relatively indigestible beta-glucan, leading to reduced feed consumption and nutrient and fat utilization (Classen et al., 1985). Therefore, supplemental beta-glucanase enzyme is required in chick diets (Campbell and Bedford, 1992).

Similar to wheat and maize, barley protein is nutritionally unbalanced for non-ruminants and requires protein supplementation (Foster and Prentice, 1987). It contains limited amounts of the essential amino acids lysine, methionine, tryptophan and threonine (Newman and Newman, 1992). However, barley protein has better metabolic availability than wheat and maize protein and requires reduced levels of supplementation to reach similar nutritional value (Whitehouse, 1973). Protein composition is less important for ruminants. Dietary nitrogen is converted into essential amino acids by rumen microorganisms and subsequently absorbed by the animal (Foster and Prentice, 1987). With higher protein levels in barley than maize, barley requires less nitrogen supplementation for ruminants (Matsushima, 1979).

Barley is ideally suited for malting for three main reasons: high enzymatic activity, a protective hull for the germinating seedling and use in filtration, and the firm texture of the steeped kernel (Burger and LaBerge, 1985). Upon germination, barley produces beta-glucanase to degrade endosperm cell walls, proteases to breakdown storage protein, and alpha and beta-amylase and limit detrinase to efficiently hydrolyze starch into fermentable sugars. Of the cereals, only wheat and rye produce similar amylolytic activity (Burger and LaBerge, 1985). However, only barley has a tightly adhering hull to assist in protection and filtering during the malting and brewing processes respectively (Burger and LaBerge, 1985).

Compared to wheat, maize and rice, barley has little application as a food crop. However, in areas where other cereals are not well adapted, barley is an important staple food (Poehlman, 1985). Interest in food barley may increase, with findings of the

hypocholesterolemic (reduction of blood cholesterol) effect of barley's soluble beta-glucan fibre in reducing heart disease (Klopfenstein and Hoseney, 1987; McIntosh et al., 1991). Barley is also reported to reduce postprandial glucose concentration in diabetics and colon cancer (Jenkins et al., 1978).

2.2 Grain Hardness

Grain hardness has been described as the resistance of the kernel to fracture (Anjum and Walker, 1991). In barley, grain hardness is gaining importance in quality determination, while the wheat industry has used it for decades to differentiate grain quality and market classes. Hard wheat kernels require more force to fracture while soft wheat grains require less energy, caused by differences in the endosperm starch-protein matrix. In wheat, hardness has been attributed to friabilin, a complex of puroindoline-a and b polypeptides that prevent starch granules from adhering to the starch-protein matrix (Hogg et al., 2004). Grain hardness has also been described as the extent of endosperm packing (Holopainen et al., 2005). Mealiness describes loosely packed cells with air spaces between starch granules while steeliness describes densely packed cells forming a dense starch-protein matrix (Chandra et al., 1999). These terms are synonymous with hardness as steely describes a hard and mealy describes a soft endosperm (Allison, 1986).

Unlike wheat, the biochemical basis of barley grain hardness is not clearly understood. Differences in protein levels, starch-protein interactions and cell wall components have all been investigated, with contradictory results. In barley, Allison et al. (1979b) and Washington et al. (2001) reported good correlation between grain hardness and total protein content, with beta-glucan being independent. In contrast, Henry and Cowe (1990) reported a positive correlation between hardness and beta-glucan ($r = 0.49$), with total protein being independent ($r = -0.10$). Furthermore, Chandra et al. (2001) found no relationship between grain hardness and protein or beta-glucan.

Researchers have attempted to identify proteins and corresponding genes responsible for starch-protein association in hard barley. In wheat, puroindoline-a and b are simply inherited polypeptides, coded for by genes linked to the "Hardness locus" on chromosome 5D (Jolly et al., 1996). Barley contains polypeptides homologous to wheat puroindolines called hordoindolines (Gautier et al., 2000), controlled by genes on the short arm of chromosome 7 (5H) (Rouves et al., 1996). Darlington et al. (2000) found significant levels of friabilin (hordoindoline) interacting

with starch granules in hard barley. However, further work by Darlington et al. (2001) found no clear relationship between hordoindoline-a and b and grain hardness. Fox et al. (2007a) also found that differences in hordoindolines did not account for variation in hardness and proposed that other gene regions may indirectly affect hardness. Chandra et al. (1999) reported γ-hordein proteins were present in steely endosperm areas but absent in mealy.

Environmental conditions during plant growth and grain maturation significantly affect grain hardness. In wheat, Stenvert and Kingswood (1977) reported that wheat grain hardness increased with higher temperatures during kernel dry down. Higher temperatures promoted endosperm cell shrinkage, protein coalescence, and starch entrapment resulting in the formation of a more continuous starch-protein matrix. Inversely, cool temperatures promoted development of mealy grains with a discontinuous protein matrix and loose open endosperm appearance. Conversely, in malting barley, Wallwork et al. (1998) found high temperatures (35°C) during development increased mealiness rather than steeliness. Stenvert and Kingswood (1977) reported that even soft grains may become compacted with surplus protein if conditions are favorable for protein synthesis.

Grain hardness appears to be a heritable trait. In barley, Allison (1986) traced the mealiness of modern British malting barley cultivars to a few older mealy European parents. Frimmel (1976) found heritability of grain hardness to be similar to grain weight while Thomas et al. (1996) reported milling energy heritability ranged from 0.55 to 0.87. Recently, Fox et al. (2007b) reported heritability of SKCS hardness greater than 0.82 in barley. In wheat, Stenvert and Kingswood (1977) found cultivars ranked similarly over environments, although the magnitude of hardness changed. Martin et al. (2001) reported high narrow-sense heritability (h^2 = 0.88) of SKCS hardness in wheat.

2.2.1 Grain Hardness Measurement

In barley, grain hardness has been studied in various ways, including Single Kernel Characterization System, Milling Energy, Scanning Electron Microscopy, Light Transmission, and Particle Size Analysis as detailed below.

2.2.1.1 Single Kernel Characterization System

Grain hardness may be determined by measuring crush force, using the Perten Single Kernel Characterization System (SKCS) developed by Martin et al. (1993). The SKCS crushes single kernels between a narrowing crescent-shaped gap and toothed rotor to obtain crush-response profiles and conductivity measurements. From 300 kernel samples, these data are algorithmically integrated to provide kernel hardness, weight, diameter, and percent moisture (Gaines et al., 1996). The standard deviation indicates the degree of sample uniformity.

Although initially developed for wheat, the SKCS has found application in other grains, including barley. In barley, Nielsen (2003) reported significant correlation between SKCS hardness and modification and friability, making it a good predictor of malt quality. In food barley, Washington et al. (2001) found the SKCS a valuable tool in identifying pearling quality and uniformity in barley for the Japanese food (rice extender, miso and shochu) market. They indicated that SKCS hardness coefficient of variation was the best predictor of pearling yield ($r = -0.78$) and broken kernels ($r = -0.77$). In feed barley, Beecher et al. (2002) found a small but significant relationship between SKCS hardness and dry matter digestibility ($r = - 0.37$) and proposed further research with application in feed quality selection.

2.2.1.2 Milling Energy

Grain hardness may also be determined by measuring milling energy (ME), using the 'Comparamill' developed by Allison et al. (1979a). Milling energy is the measurement of electrical energy required to mill small (five gram) grain samples into flour (Allison et al., 1976). As samples are milled, the deceleration in a rotating flywheel driving the mill hammers is recorded and equated to ME (joules).

Milling energy is predominantly applied to barley for malting. Allison et al. (1976) established that milling energy differentiates between good and poor malting barley varieties, with good malting varieties requiring less ME. Allison et al. (1979a) reported that ME was negatively correlated with hot water extract ($r = -0.76$). Henry and Cowe (1990) reported ME negatively correlated with extent of modification ($r = -0.56$) with lower ME indicating more modification.

2.2.1.3 Scanning Electron Microscopy

Grain hardness has been investigated using Scanning Electron Microscopy (SEM). Scanning Electron Microscopy of the endosperm cells allows visual observation of the starch-protein matrix and compaction characteristics.

Using SEM, Palmer (1991) noted a gradient where outer endosperm areas were compact with dense layers of protein surrounding starch, whereas inner areas were open with little protein surrounding starch. He also studied barley samples accepted for malt that caused brewhouse problems and identified discrete endosperm areas that resisted modification, due to reduced hydration and enzyme movement. Using SEM, Brennan et al. (1996) observed that good malting barley had limited starch-protein association in all endosperm areas. They also noted that upon fracturing, starch and protein easily separated in good malting barley but remained firmly connected in poor, increasing starch damage. Similar to Palmer, Chandra et al. (1999) found various random dense (steely) endosperm areas that restricted water and enzyme movement. They proposed a quantitative measurement of the endosperm, such as light transmission, which may better describe the overall grain structure, as detailed below.

2.2.1.4 Light Transmission

Grain hardness in barley may be determined by measurement of light transmission, using the Light Transflectance Meter (LTm) developed by Brewing Research International (Chandra et al., 2001). The LTm measures the average quantity of laser light passing through whole grains (97 seeds sampled), with low LTm values (<200 mV) indicating mealy grain texture and high (>300 mV) indicating steely. Woonton et al. (2003) reported a significant negative correlation between LTm values and malt homogeneity ($r = -0.80$). Using the LTm to differentiate endosperm texture, Holopainen et al. (2005) found an association between texture and malting performance, with steely grains being less friable and slower to modify. Also using the LTm, Moss and Givens (2002) reported a relationship between endosperm texture, grain hardness and nutritive value of wheat (rumen degradable starch) for ruminants.

Light transmission based grain hardness has also been measured with image analysis software. Erasmus and Taylor (2004) captured images of illuminated maize endosperms and quantified the light transmitted to report a strong positive correlation between transmission and vitreousness ($r = 0.81$). In durum wheat, Xie et al. (2004) used digital analysis of light

transmission and reflection images to differentiate vitreous and non-vitreous kernels. Compared to visual inspection, combining light transmission and reflection correctly classified 94.1% of vitreous and 85.6% of non-vitreous kernels. In barley, Nielsen (2003) measured total light reflectance of kernels using the GrainCheck^{TM} 310 instrument (FOSS Tecator AB, Höganas, Sweden) along with the SKCS hardness to predict malting quality. He reported these two factors were each significantly positively correlated with malt beta-glucan (r = 0.74), wort beta-glucan (r = 0.82), friability (r = 0.82), and wort viscosity (r = 0.71).

2.2.1.5 Particle Size Analysis

Particle Size Analysis (PSA) is the measurement of particle size after grain milling or feed processing, and is a long-used method to evaluate end-use quality. It is important to note that milled flour particle size is measured at the micron level rather than the larger millimeter sieves used in feed analysis.

Flour particle size can be used to identify hard or soft wheat, which determines end-use suitability (Morris and Rose, 1996). Soft wheat fractures easily into flour with small particle size and limited starch damage, while hard wheat produces larger flour particles with increased starch damage. Similarly in maize, hard endosperm mills into larger particles than soft endosperm (Eckhoff and Paulsen, 1996). Although not significant, Ramsey et al. (2001) reported a negative trend between feed particle size and grinding time (Brabender kernel hardness measurement) in hulled barley (r = -0.50), with soft barley having longer grinding times and smaller feed particle size.

Researchers have found that ruminant performance is significantly influenced by particle size of the feed consumed. Galyean et al. (1981) reported a negative relationship between feed particle size and rumen dry matter disappearance of maize and sorghum. Similar results for maize and barley were reported by McAllister et al. (1993) and for barley by Bowman et al. (1996).

2.3 Extent and Rate of Dry Matter Disappearance in Ruminants

Dry matter disappearance (DMD) can be described as the rate and/or extent of feed breakdown in the rumen (Orskov et al., 1969; Mehrez et al., 1977). Measuring DMD is critical for optimizing feeding practices, especially in cereal rich diets high in available energy. Feeding

high concentrations of cereal grains to ruminants can lead to maladies including acidosis, bloat and liver abscesses as reviewed by Galyean and Rivera (2003).

Measuring DMD of cereals also has application in optimizing rumen bypass starch for degradation in the small intestine. Owens et al. (1986) described ruminally degraded starch as being only 70% as efficient as compared to starch degraded in the small intestine, with energy being lost through fermentation heat, methane and excreta. However, they noted that reduced rumen digestion in turn may decrease the microbial protein supply for the animal. Taniguchi et al. (1995) recommended reducing rumen bypass starch to provide the greatest energy and protein for the animal, unless starch and protein escape rumen fermentation jointly.

2.3.1 DMD Factors

Dry matter disappearance of cereals is dependent on complex, interrelated factors, including feed composition, consumption over time, mechanical and chemical alterations of the feed, and adaptation of rumen microorganisms (Huntington, 1997). The following is a brief review of DMD as it relates to feed composition of cereal species and varieties and mechanical alteration of feed particle size.

2.3.1.1 Cereal Species

Cereal species differ in DMD and its relation to starch digestion in the rumen. Orskov (1986) summarized several studies and concluded that at least 90% of barley and wheat starch is fermented in the rumen, compared to about 60% for maize. This slower digestion of maize is preferred to avoid health problems, such as acidosis, bloat and liver abscesses (Orskov, 1986).

Determining the cause of differences between cereal species has been the focus of several reports. McAllister et al. (1990) examined microbial colonization of maize, sorghum, barley and wheat and found differences in bacterial attachment to starch granules. Coccoid bacteria primarily colonized maize starch granules while numerous species colonized those of other cereals. Subsequent work by McAllister et al. (1993) indicated DMD differences between species may be caused by endosperm protein and structural components rather than the starch granules themselves. Their research with isolated barley and maize starch granules showed no differences in rate or extent of rumen digestion. However, the horny endosperm protein associated with starch granules in maize was extremely resistant to microbial attack, compared

with rapidly degraded barley protein. Zein is the major component of horny maize endosperm protein (Christianson et al., 1969) and has similarly been reported to resist microbial degradation (Ely et al., 1967). Differences in degradation may even exist between barley protein classes. Beecher et al. (2002) reported that genotypes with hordoindoline alleles associated with harder grain also tended to result in lower dry matter digestibility.

2.3.1.2 Cereal Variety

Variation in DMD also occurs between types within the same cereal species. Michalet-Doreau and Champion (1995) reported the extent of rumen starch disappearance was lower in vitreous maize (58.0%) compared to floury maize (71.0%). Moreover, vitreous maize had significantly less rapidly degradable material than floury maize (19.4% vs. 32.8%). In wheat, Moss and Givens (2002) reported differences in extent of rumen degradable starch among varieties varying in starch content, grain hardness and endosperm texture.

In barley, Bowman et al. (2001) examined some 1500 barley accessions from the USDA National Small Grains Collection and found substantial variability in extent of DMD after 3 hours, ranging from 8.2 to 62.1%. They reported that extent of DMD was lower in six-row barley versus two-row. This contrasts with earlier findings by Boss and Bowman (1996a) who reported no differences in extent of DMD between the two-row barley varieties Harrington and Gunhilde and the six-row barley variety Medallion. However, Boss and Bowman (1996a) found faster average daily gain in steers fed Harrington malting barley versus Gunhilde and Medallion feed barley varieties. Similarly, Ovenell-Roy et al. (1998) found improved feed to gain ratios for steers fed two-row Harrington malting barley compared to the six-row feed varieties Boyer, Hesk and Steptoe.

In a study with twenty-two Canadian barley cultivars, Lehman et al. (1995) found no differences in rate of DMD among two-row, six-row, hulled, hulless, malt and feed varieties. However, they found feed barley varieties contained 4% more slowly degradable fraction compared to malting types, while hulless varieties contained 11% more than hulled. Similarly, Yu et al. (2003) found no difference in rate of DMD between two-row varieties Harrington (malt) and Valier (feed). Nonetheless, the effective degradation of dry matter (EDDM) was lower for Valier than Harrington, indicating a delay in the onset of Valier's fermentation.

2.3.1.3 Feed Particle Size

Feed particle size influences DMD primarily because of differences in exposed surface area per unit volume with larger particles having comparatively less area exposed. As microbial attachment to particles is essential for rumen fermentation (McAllister et al., 1994), larger particles with less exposed area per volume will degrade more slowly. Studies by Walker et al. (1973) and Galyean et al. (1981) confirmed that smaller particles disappear more quickly in the rumen. However, particles smaller than a critical size (approximately 1.0-mm) typically flow out of the rumen without being fermented (Poppi et al., 1980). Conversely, whole grains may also travel through the animal potentially undigested (Orskov, 1986) due to difficulties in microbial penetration of the fibrous outer layers (especially the case for hulled barley).

Tempering barley before processing can increase particle size and reduce the proportion of fines (particles less than 1.0-mm) (Hironaka, 1981 as cited by Hironaka et al., 1992). Hironaka (1981) found steers fed tempered rolled barley had an increased average daily gain and lower feed per unit gain compared to dry-rolled barley. However, other reports have suggested that tempering shows no improvement in animal performance (Bradshaw et al., 1996 and Mathison et al., 1997).

Achieving feed particle size that maximizes feed efficiency while maintaining animal health can be challenging. Current industry recommendations are that dry barley be processed with less than 5% whole kernels remaining (Mathison et al., 1997) and less than 3% passing through a 1.0-mm screen (fines) (Mathison, 2002).

2.3.2 Breeding for Improved Feed Barley Quality for Ruminants

Conventional breeding of feed barley varieties for ruminants has relied on selection for physical grain characteristics, such as bulk density, kernel weight and plumpness (Ullrich, 2002). However, reports by Coates et al. (1977) and Mathison et al. (1991) have indicated that bulk density does not correlate well with feeding value, particularly for a relatively narrow range found in more dense barley samples. Ramsey et al. (2001) reported that bulk density explained only 48% of the variability for *in vivo* total tract dry matter digestibility of hulled barley varieties. Mathison et al. (1991) reported that more dense barley degraded slower than less dense barley *in vitro* but with no differences in average daily gain, dry matter intake or carcass quality.

Selection for larger feed particle size and lower acid detergent fiber (ADF) to improve feed barley for ruminants has been suggested by Bowman et al. (1996) and Surber et al. (2000). Surber et al. (2000) reported that extent of rumen DMD was inversely correlated to mean feed particle size (r = -0.57) and ADF content (r = -0.81). LB13 and LB30, two recombinant inbred lines (RILs) developed from a cross between Lewis (two-row malt) and Baronesse (two-row feed) barley varieties were selected based on good agronomic performance, larger particle size after rolling, lower ADF content and lower *in situ* extent of DMD (Surber et al., 1999). In a feedlot trial, steers fed LB13 and LB30 showed an 8.6% (1.72 kg/d) and 10% (1.74 kg/d) increase in average daily gain, respectively, compared to parent varieties Lewis (1.56 kg/d) and Baronesse (1.61 kg/d) (Boss et al., 1999). In 1999, LB30 was registered by Montana Agricultural Experiment Station as the variety 'Valier' (Blake et al., 2002).

During malting, soft barley modifies more easily as enzymes are more able to attack the starchy endosperm. Conversely, hard barley has incomplete or delayed endosperm modification. It is the hypothesis of this research that hard barley would degrade more slowly in the rumen due to more difficult microbial access to the barley starch in the rumen. The goal is to determine an efficient grain hardness evaluation technique that could be used to screen large numbers of lines in a breeding program for slow DMD rate.

The objectives of this thesis research were to: examine the effect of grain hardness, variety and environment on dry matter disappearance rates; identify accurate and efficient grain hardness selection tools for plant breeding; and study environmental effects, inheritance and heritability of grain hardness.

3. EVALUATION OF BARLEY CVS VALIER AND TR253 *IN SITU* DRY MATTER DISAPPEARANCE AND GRAIN HARDNESS ACROSS ENVIRONMENTS

3.1 Introduction

Barley grain is an important source of energy and protein for ruminant animals. However, feeding must be carefully managed to avoid acidosis, liver abscesses and bloat caused by the rapid breakdown of barley starch in the rumen. The development of slower degrading barley for ruminants may alleviate health problems associated with consumption of barley grain. Feedlot studies by Boss et al. (1999) led to the identification of a barley genotype with slower dry matter disappearance rate (DMDR) and improved cattle-feeding characteristics. Blake et al. (2002) released this genotype as the feed variety 'Valier'.

The reduced rate of starch disappearance for Valier barley may be related to endosperm hardness. Past research with malting barley has indicated that soft (mealy) endosperm rapidly modifies into malt as enzymes easily access starch granules (Palmer and Harvey, 1977). Conversely, hard barley has less effective enzymatic activity and poor malt quality. Therefore, in feed barley, selection for hard endosperm may result in slower starch degradation and improved feed quality.

In a breeding program, grain quality characteristics may be influenced by environmental factors, hampering accurate phenotypic selection. Before beginning selection, it is important to determine the effect of environment on the trait and possible genotype by environment interaction. To determine the effect of environment on DMDR, *in situ* DMD rate was measured for Valier and the barley breeding line TR253 as grown at four Western Canadian sites in 2004. Valier and TR253 were chosen as they are the parents of a double haploid population available for additional, more detailed study.

The objectives were to:

1) determine differences for *in situ* DMDR and grain hardness between Valier and TR253;
2) determine the effect of environment on DMDR of Valier and TR253; and

3) examine the endosperm ultrastructure of Valier and TR253 using Scanning Electron Microscopy.

3.2 Materials and Methods

3.2.1 Grain Sample Production

Valier and TR253 were grown in multi-site field trials at six Western Canadian sites during 2004, which were Brandon, Saskatoon (GD), Saskatoon (KCRF), Saskatoon (SF), Wakaw and Watrous. Saskatoon sites differed in seeding date and soil type and were considered distinct from each other. Valier is a two-row feed barley cultivar, released by the Montana Agricultural Experiment Station (Bozeman, USA) for its good agronomic performance and improved cattle-feeding characteristics, specifically slower DMDR. TR253 is a malting barley breeding line developed at the Agriculture and Agri-Food Canada (AAFC) Brandon Research Centre (Brandon, Canada) with good agronomic performance and malting quality.

Grain was harvested after physiological maturity and air-dried until less than 14.5% moisture for proper storage. Entries were sieved to increase relative uniformity. Samples were sieved such that only seed passing through a 3.1 x 18.75-mm slotted sieve and remaining on a 2.9 x 18.75-mm slotted sieve were retained for further evaluation.

3.2.2 Grain Processing Methods

Five hundred gram samples were minimally dry rolled (roller gap size 1.7-mm) using a Sven Roller Mill (Apollo Industries, Saskatoon, Canada) with 8-inch diameter rolls and a 1.5-horsepower motor.

3.2.3 Grain Analysis

3.2.3.1 SKCS Hardness

Three hundred whole seed per sample were evaluated using the SKCS 4100 (Perten Instruments, Springfield, IL) to determine SKCS hardness. SKCS hardness values range from 0 to 100, with 0 indicating softest and 100 hardest.

3.2.3.2 Protein

Whole seed samples were analyzed for protein content using Near Infrared Transmittance (NIT) (Infratec 1255 Food and Feed Analyzer™, Tecator AB, Höganas) based on the FOSS two-row barley protein calibration.

3.2.3.3 Feed Particle Size Analysis

A fifty-gram dry rolled sample was sieved for three minutes using a W.S. Tyler Sieve Shaker system (Mentor, OH) equipped with five brass wire sieves (3.35-mm, 2.36-mm, 2.00-mm, 1.40-mm, 1.00-mm). Material remaining above individual sieves was divided by the total weight to determine percentages of feed particle size (% 3.35-mm, % 2.36-mm, % 2.00-mm, % 1.40-mm, and % 1.00-mm). Material passing through the 1.00-mm sieve was divided by the total weight to determine the percent less than 1.00-mm (% <1.00-mm). Mean particle size (μm) of rolled grain was calculated as described by Fisher et al. (1988) with assistance from J.G. Bowman (Montana State University).

3.2.3.4 Scanning Electron Microscopy

Three seed per sample were cut transversely and mounted on electron microscope stubs, with the central endosperm section facing upward. Samples were sputter-coated with gold particles using an Edward S150B Sputter Coater (UK). Endosperm samples were examined using a SEM505 scanning electron microscope (Philips, Holland) at 3,000X magnification. Images of the prismatic and central endosperm cells from middle and central endosperm areas, respectively, were photographed using Polaroid 665 camera film.

3.2.4 *In situ* DMD Testing

Valier and TR253 grain samples from four locations grown in 2004 (Brandon, Saskatoon (KCRF), Saskatoon (SF), Wakaw) were chosen for *in situ* evaluation based on differences in SKCS hardness (Table 3.1). Valier had consistently higher SKCS hardness values than TR253 at all sites. Saskatoon (KCRF) had the highest hardness values on average, with Brandon and Saskatoon (SF) having the lowest. Saskatoon (GD) and Watrous showed minimal hardness differences between Valier and TR253 and were not chosen for *in situ* evaluation.

Table 3.1. SKCS hardness of Valier and TR253 grown at six Western Canadian sites in 2004.

	SKCS Hardness						
		Saskatoon					
Genotype	Brandon	GD	KCRF	SF	Wakaw	Watrous	Genotype Mean*
Valier	50.9	38.1	59.1	50.3	53.0	47.9	49.9 $_a$
TR253	46.1	37.8	54.9	45.8	49.6	45.2	46.6 $_b$
Site Mean	48.5	37.9	57.0	48.1	51.3	46.5	48.2 (0.67)

*Values in the same column followed by the same subscript do not differ (P<0.05). Number in parenthesis is standard error of genotype mean.

In situ DMD was determined using the nylon bag technique described by Orskov (1992). Two cannulated 1200-kg Angus steers were housed in 25-m^2 pens at the Livestock Research Building, University of Saskatchewan, Saskatoon, Canada. Animal diets were gradually adjusted to high concentrate over a six-week period before sample testing. The final diet consisted of 85% rolled barley (DM), 9% barley silage (DM), and 6% vitamin premix. The steers were fed a 5-kg ration twice daily (after bag placement) at 0800 and 1600 h with water available in pens. Care for the animals followed guidelines described in the CCAC (1993).

Seven-gram rolled barley samples were placed in 10-cm x 20-cm pre-weighed nylon mesh bags (41-µm mesh size). According to a 'gradually in/all out' time schedule (0, 2, 4, 8, 12, or 24 hours), mesh sample bags were placed in the ventral rumen sac inside a larger 30-cm x 40-cm mesh lingerie bag (with 1-m attached rope and weight). After incubation, all bags were removed from the rumen and immediately rinsed with water to remove coarse rumen material. Bags were further washed six times, with each wash consisting of a one-minute agitation using 100-ml water/bag, until rinse water became clear. Samples of 0-hour time-period were washed using the above protocol, without rumen incubation. Bags were dried for 48 hours at 50°C and weighed. Sub-samples were oven dried to zero moisture for final weight correction.

Arranged as a Completely Randomized Design, entries were replicated per time-period with 15 bags per entry and placed in the rumen of each steer over two days. Samples of 0, 2, 4, 8-hour time-periods were duplicated, while 12 and 24-hour samples were replicated 3 and 4 times, respectively. The experiment was repeated four times (runs). Run 2 was completed with only one steer over three days after excessive rumen fluid drained from the second steer during bag placement. Run 3 was completed over four days with one steer as the second steer was slightly bloated before initial bag placement.

3.2.5 Rumen Degradation Analysis

Rumen degradation analysis of dry matter was determined using the first-order kinetics equation described by Orskov and McDonald (1979). The equation $R(t)=B+C+\exp(-Kd*(t-T_0))$ describes R(t) as the residual incubated material after t hours of incubation (g/kg), B as the potentially degradable fraction (g/kg), C as the undegradable fraction (g/kg), Kd as the degradation rate (h^{-1}) and T_0 as the lag time before degradation (h). The results were obtained using the PROC NLIN procedure of SAS (2005) for least-squares estimation of nonlinear models (Gauss-Newton).

Effective degradation of dry matter (EDDM) (g/kg) was calculated by the formula: $EDDM=A+B*K_d/(K_d+K_p)$, where A (g/kg) describes the rapidly soluble fraction removed after 0-h washing. K_p describes the assumed rumen content passage rate of 0.06 h^{-1} (Tamminga et al., 1994). Rumen undegraded dry matter (RUDM) (g/kg) was calculated by the formula: $RUDM=C+B*K_p/(K_p+K_d)$ with assumed rumen content passage rate of 0.06 h^{-1}.

3.2.6 Statistical Analysis

Differences between genotypes for SKCS hardness, protein, and feed particle size were analyzed using PROC TTEST with sites as paired observations ($P<0.05$). To test differences in genotype and environment, *in situ* data was initially analyzed using PROC GLM in SAS (SAS Institute, Cary, NC, USA, 2006) as a 2 genotype X 4 environment factorial with $P<0.05$ significance level. Because no genotype or environment differences were detected, further analysis of genotype differences at individual sites was performed using the paired observation PROC TTEST ($P<0.10$). Significance level of 0.10 was chosen to test differences at individual sites because of large differences but higher variability between runs at Saskatoon (SF) site.

3.3 Results

3.3.1 Grain Analysis Results

Analysis of SKCS hardness, protein and feed particle size of Valier and TR253 revealed no differences between the lines for protein or feed particle size ($P>0.11$) (Table 3.2). Significant differences were detected for SKCS hardness ($P<0.0006$), with Valier having harder grains than TR253 (53.3 versus 49.1, respectively) (Table 3.2).

Table 3.2. Genotype and environment SKCS hardness, protein and feed particle size means of Valier and TR253, grown at Brandon, Saskatoon (KCRF and SF) and Wakaw sites in 2004.

	Genotype				Environment			
						Saskatoon		
Trait	Valier	TR253	SE	Pr>t	Brandon	KCRF	SF	Wakaw
SKCS Hardness	53.3_a	49.1_b	0.28	0.0006	48.5	57.0	48.1	51.3
Protein (%)	11.3	10.7	0.27	0.12	10.2	11.3	11.1	11.5
% Particles > sieve size (mm)								
3.35	26.6	22.0	3.91	0.32	20.9	34.8	16.1	25.6
2.36	48.8	50.1	1.74	0.52	53.0	45.3	52.4	47.2
2.00	13.3	14.9	1.74	0.43	14.0	11.2	16.5	14.8
1.40	6.59	7.93	0.71	0.16	6.88	5.15	9.39	7.62
1.00	2.06	2.34	0.12	0.11	2.24	1.59	2.67	2.31
<1.00	2.64	2.78	0.17	0.46	3.12	2.11	3.04	2.58
Mean Particle Size (μm)	3033	2831	149	0.27	2833	3336	2631	2929

Values in the same row followed by the same subscript do not differ (P<0.05). Environment values based on one replication only.

Environment means of Valier and TR253, grown at Brandon, Saskatoon (KCRF and SF) and Wakaw is given in Table 3.2. SKCS hardness was highest at Saskatoon (KCRF) (57.0) site, followed by Wakaw, Brandon and Saskatoon (SF) (51.3, 48.5, 48.1, respectively) (Table 3.2). Similarly, the Saskatoon (KCRF) site had the largest percentage of particles >3.35-mm and mean particle size after rolling, with Wakaw, Brandon and Saskatoon (SF) following (Table 3.2). Brandon had the largest percentage of particles <1.00-mm compared to Saskatoon (SF), Wakaw and Saskatoon (KCRF). Protein content was similar at Saskatoon (KCRF and SF) and Wakaw (11.1 to 11.5%), while protein was lowest at Brandon at 10.2% (Table 3.2).

3.3.2 Scanning Electron Microscopy

Differences were observed using Scanning Electron Microscopy (SEM) between Valier and TR253 endosperm ultrastructure (Figure 3.1 to 3.8). However, differences were not consistent across growing location. In prismatic endosperm cells from Brandon (Figure 3.1), Valier A and B-type starch granules (3.1A) were extensively enveloped in a sheet of starch-associated protein, resulting in an almost continuous starch-protein matrix. Individual endosperm cells were indiscernible. Comparatively, TR253 A and B-type granules (3.1B) were

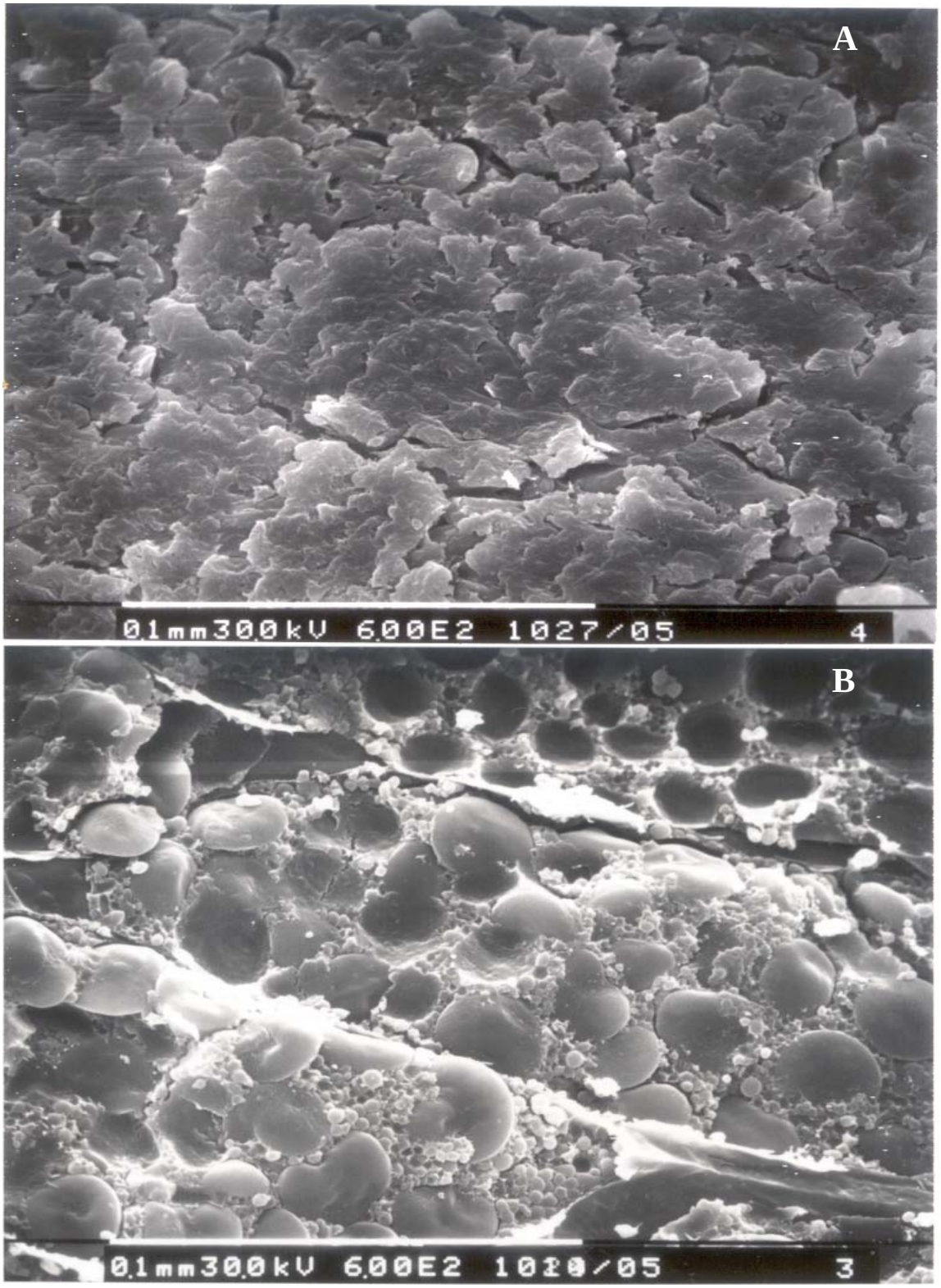

Figure 3.1. Scanning Electron Micrographs of Valier (A) and TR253 (B) prismatic endosperm illustrating the starch-protein matrix of the genotypes as grown at Brandon in 2004.

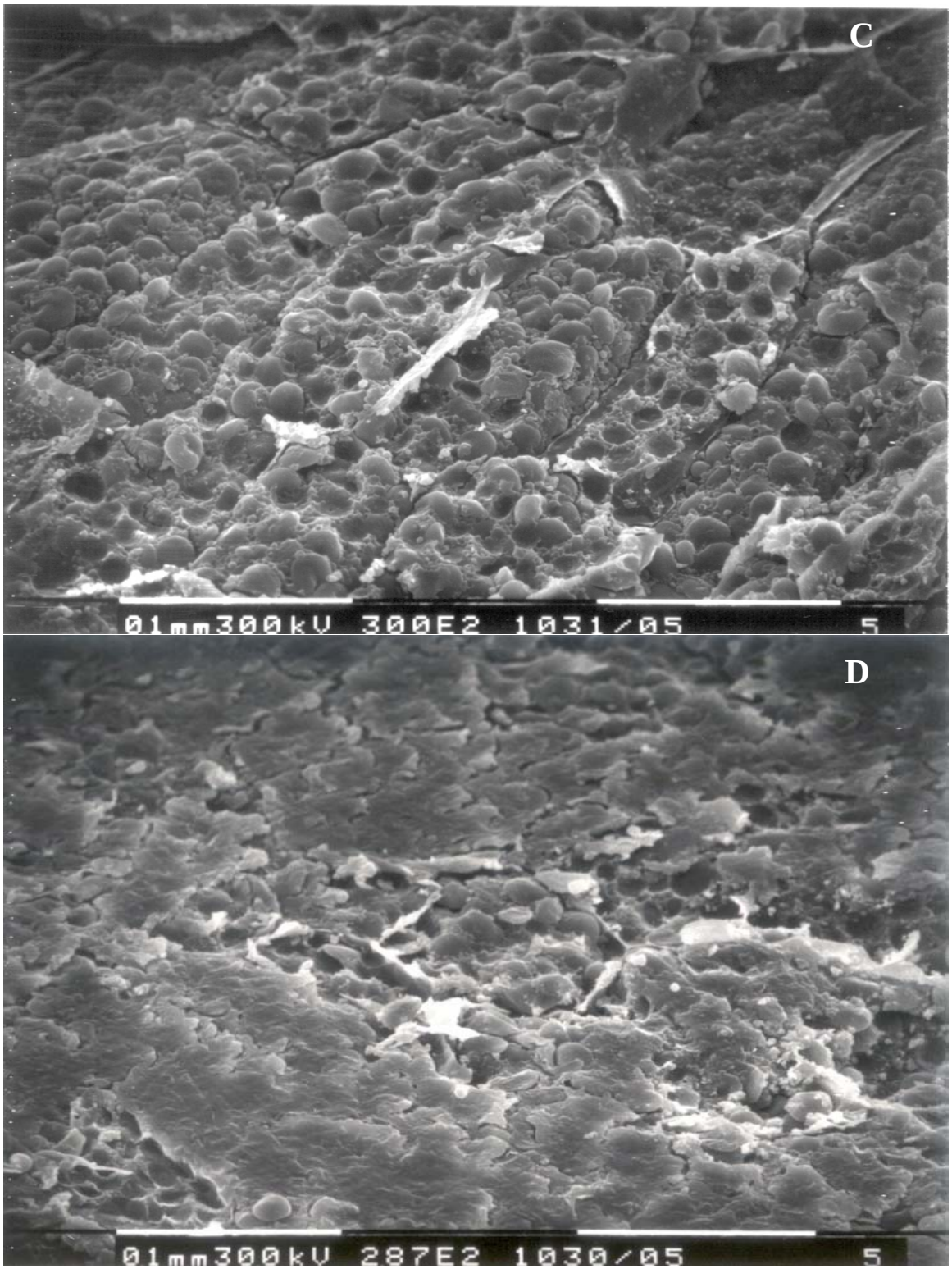

Figure 3.2. Scanning Electron Micrographs of Valier (C) and TR253 (D) prismatic endosperm illustrating the starch-protein matrix of the genotypes as grown at Saskatoon (KCRF) in 2004.

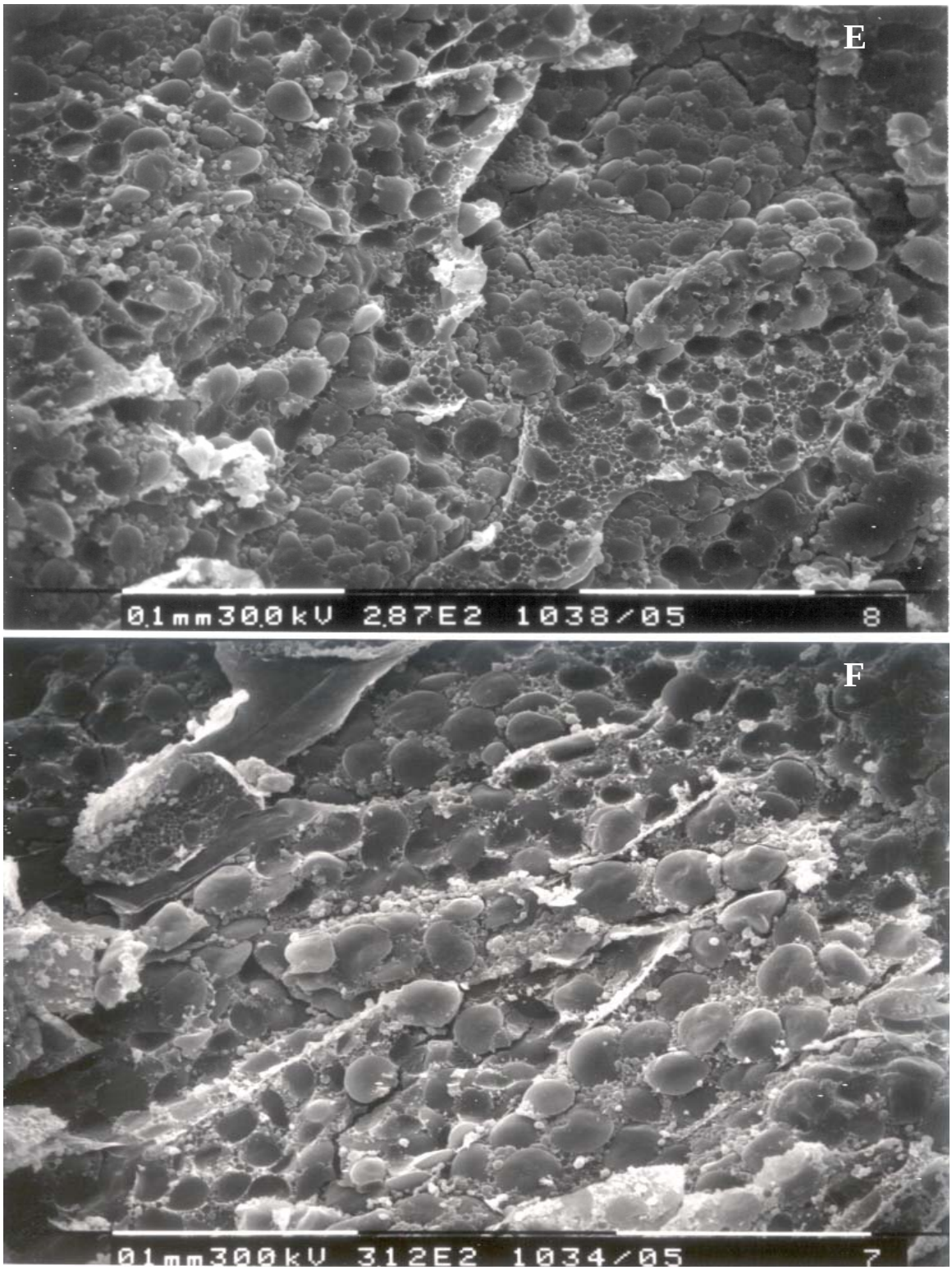

Figure 3.3. Scanning Electron Micrographs of Valier (E) and TR253 (F) prismatic endosperm illustrating the starch-protein matrix of the genotypes as grown at Saskatoon (SF) in 2004.

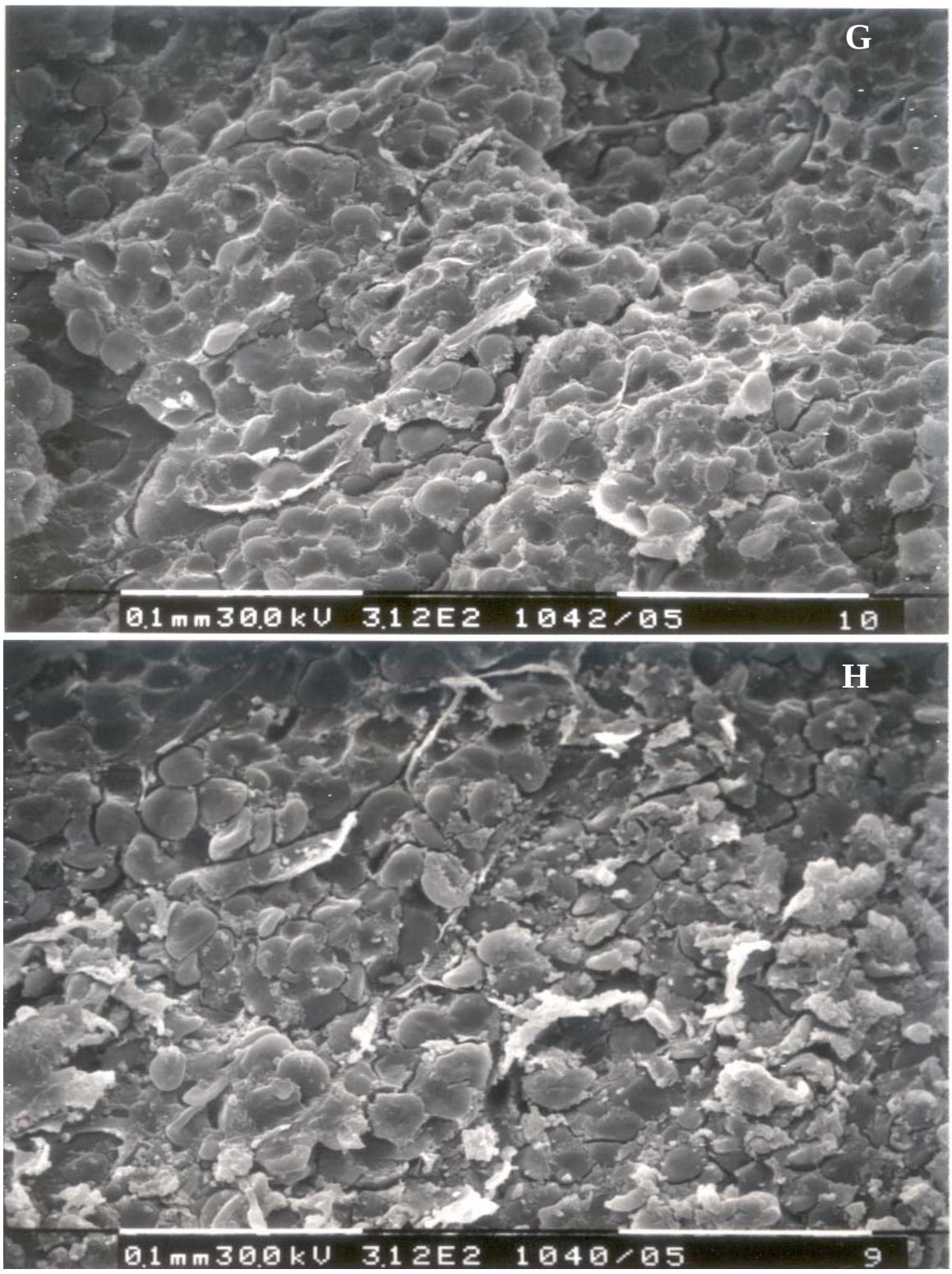

Figure 3.4. Scanning Electron Micrographs of Valier (G) and TR253 (H) prismatic endosperm illustrating the starch-protein matrix of the genotypes as grown at Wakaw in 2004.

readily exposed, with minimal associated protein surrounding them. Individual cells were easily distinguishable. However, in other middle endosperm areas of TR253, pockets of cells with starch-associated protein were identified (not shown). These areas had less starch-associated protein than the corresponding Valier matrix as B-type granules were dislodged from the TR253 matrix and not from Valier.

Conversely, in samples from Saskatoon (KCRF) (Figure 3.2), TR253 (3.2D) starch was extensively coated with protein, masking A and B-type granules and individual endosperm cells. Valier's endosperm cells were readily distinguishable and appeared intact, with minimal disruption of A and B-type granules (3.2C). Protein coating was not evident.

In samples from Saskatoon (SF) (Figure 3.3), minimal differences in the starch-protein matrix were noted between Valier (3.3E) and TR253 (3.3F). Both had limited starch-protein association with B-type granules dislodged from the cells. Individual cells were readily apparent.

In samples from Wakaw (Figure 3.4), Valier endosperm cells (3.4G) appeared intact with minimal granule disruption. Granules were extensively enveloped in protein, resulting in an almost continuous starch-protein matrix. Comparatively, TR253 (3.4H) had more cellular disruption as B granules were dislodged from the starch-protein matrix. Patches of cells with starch-associated protein were scattered throughout the middle endosperm (not shown).

In central endosperm areas (Figure 3.5 to 3.8), differences were also observed between Valier and TR253 endosperm ultrastructure. Again, differences were not consistent across growing location. In samples from Brandon, central endosperm areas (Figure 3.5) of Valier (3.5A) had large amounts of starch-associated protein and a compact structure, whereas TR253 (3.5B) endosperm was more open with loose A-type granules. Protein coating was evident in some areas, but less than Valier. Similarly for Saskatoon (KCRF) (Figure 3.6) and Wakaw samples (Figure 3.8), Valier (3.6C and 3.8G) A-type granules were noticeably coated with protein and densely packed into the cells, in contrast with TR253 (3.6D and 3.8H), which had a more open structure and loose granules. However, from Saskatoon (SF) (Figure 3.7), TR253 (3.7F) samples appeared compact with more protein-associated starch granules compared to Valier (3.7E), which had an open structure with limited protein sheeting. Few B-type granules were present in any central region.

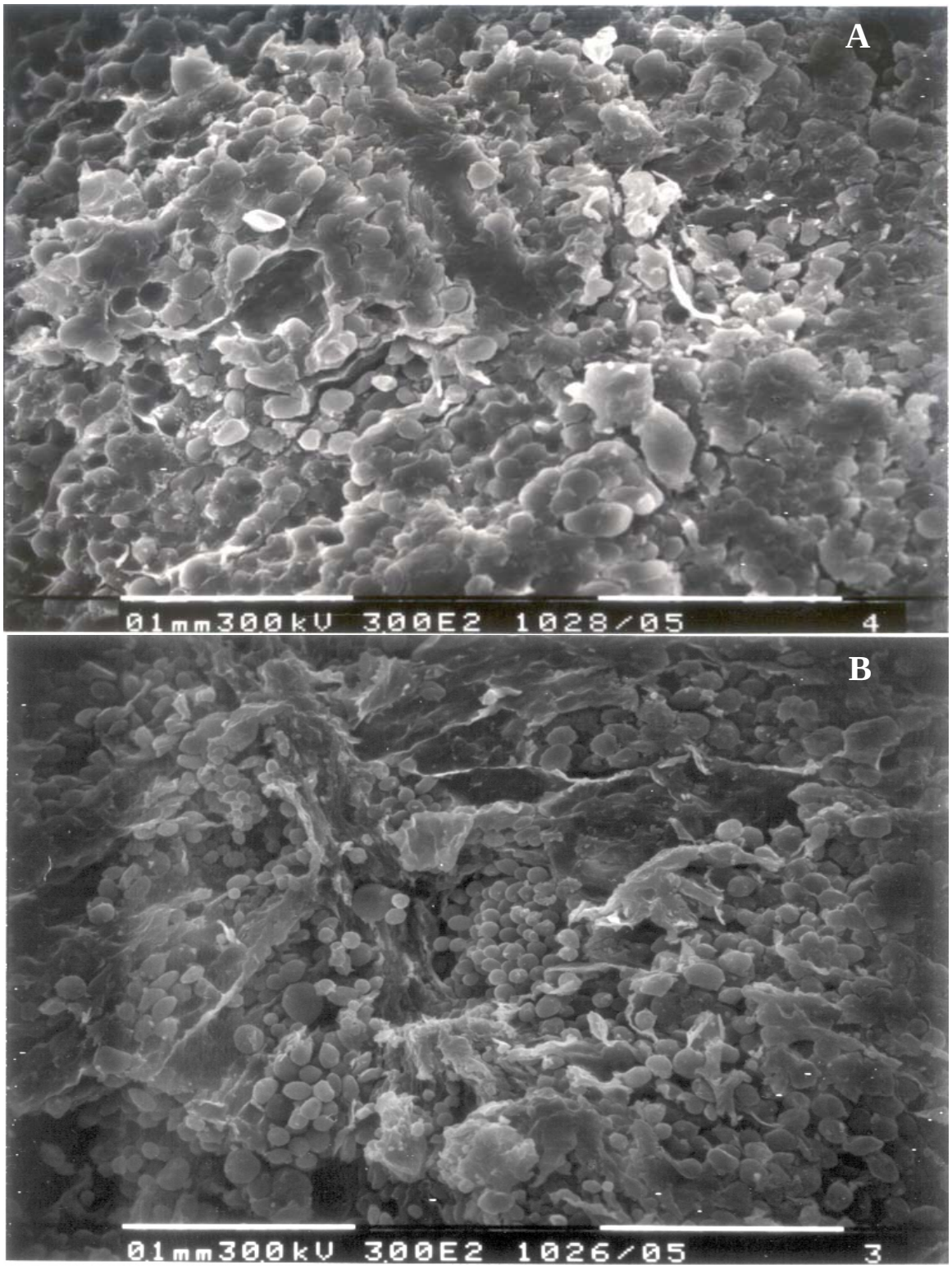

Figure 3.5. Scanning Electron Micrographs of Valier (A) and TR253 (B) central endosperm illustrating the starch-protein matrix of the genotypes as grown at Brandon in 2004.

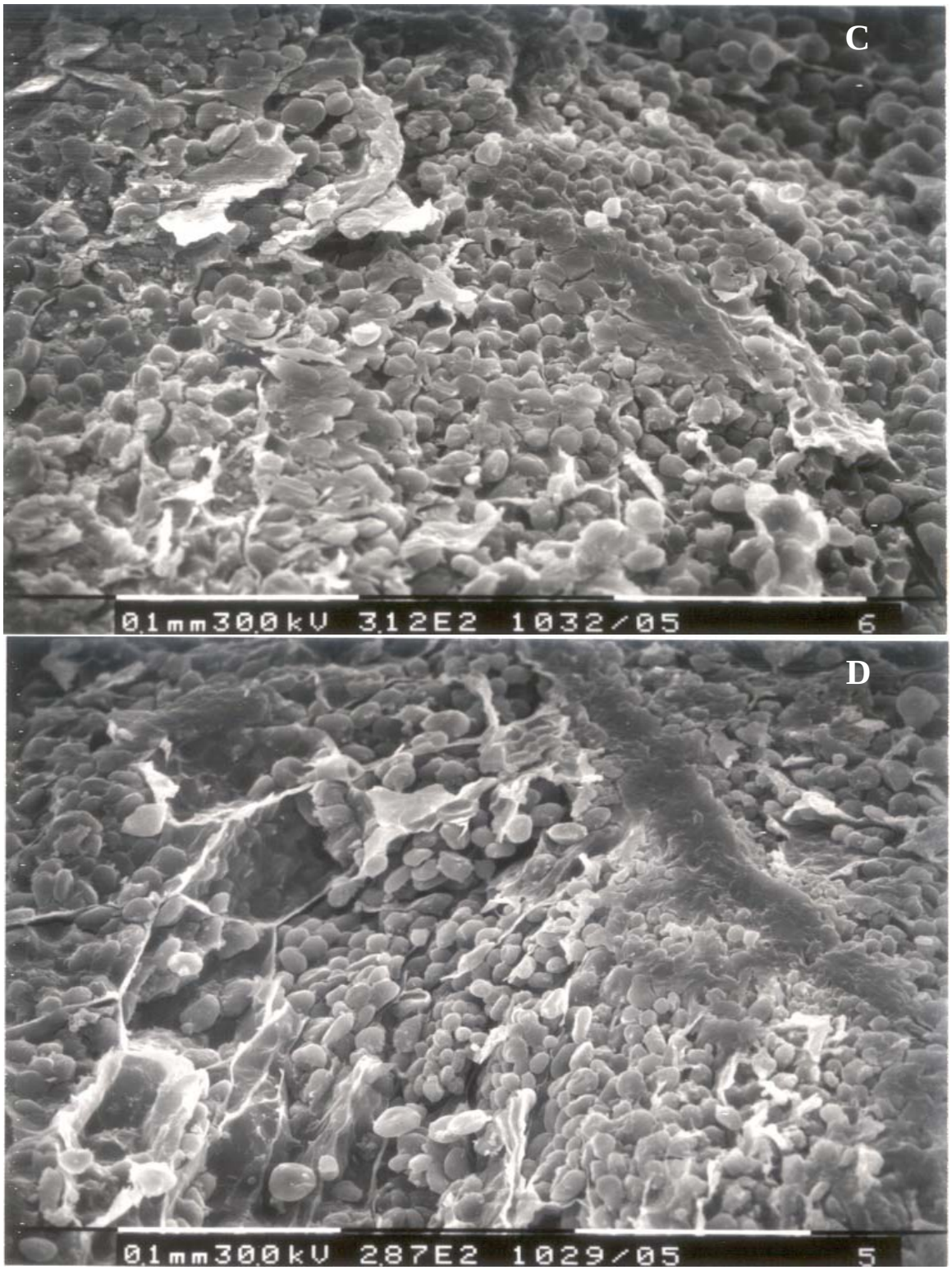

Figure 3.6. Scanning Electron Micrographs of Valier (C) and TR253 (D) central endosperm illustrating the starch-protein matrix of the genotypes as grown at Saskatoon (KCRF) in 2004.

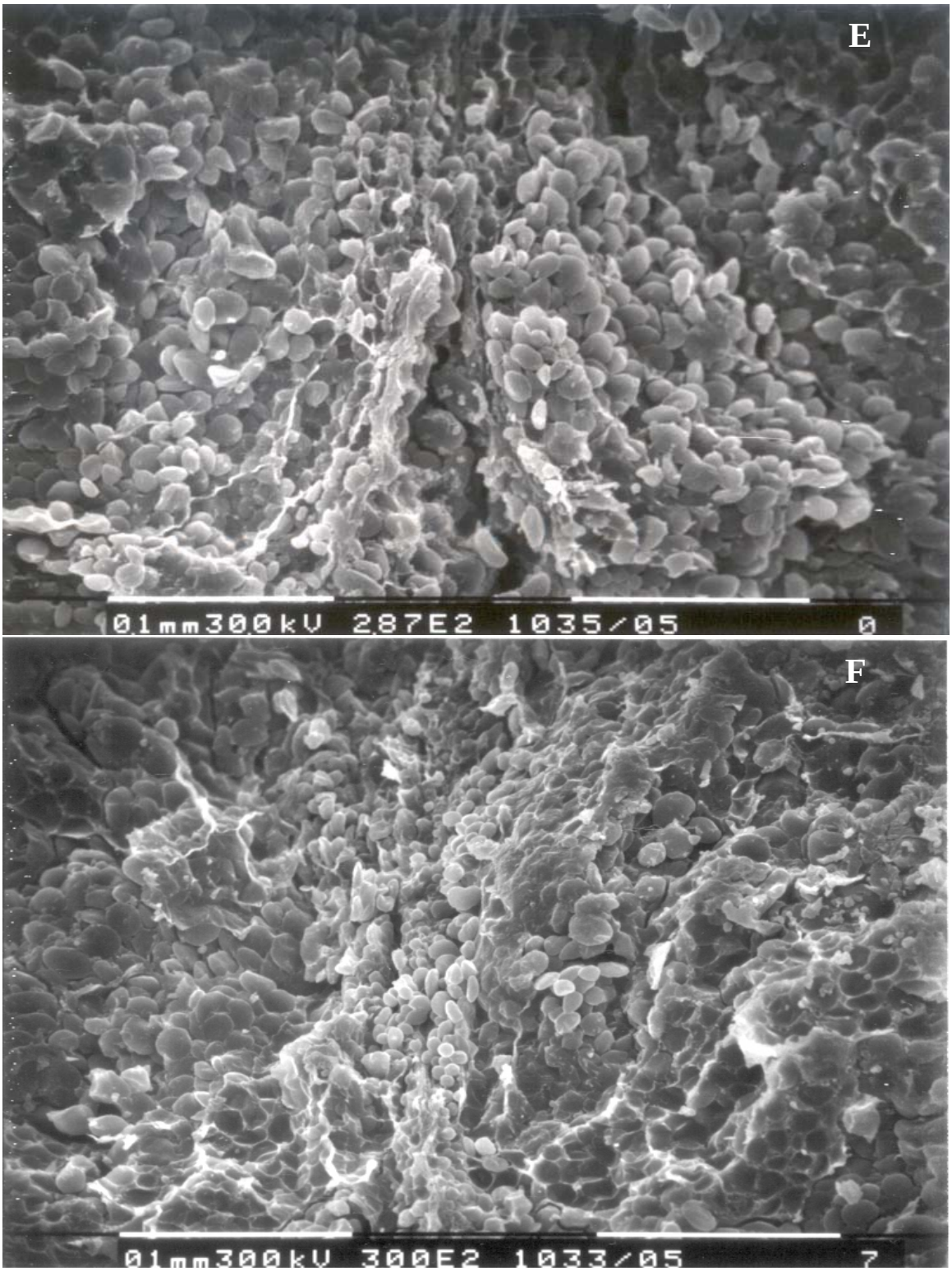

Figure 3.7. Scanning Electron Micrographs of Valier (E) and TR253 (F) central endosperm illustrating the starch-protein matrix of the genotypes as grown at Saskatoon (SF) in 2004.

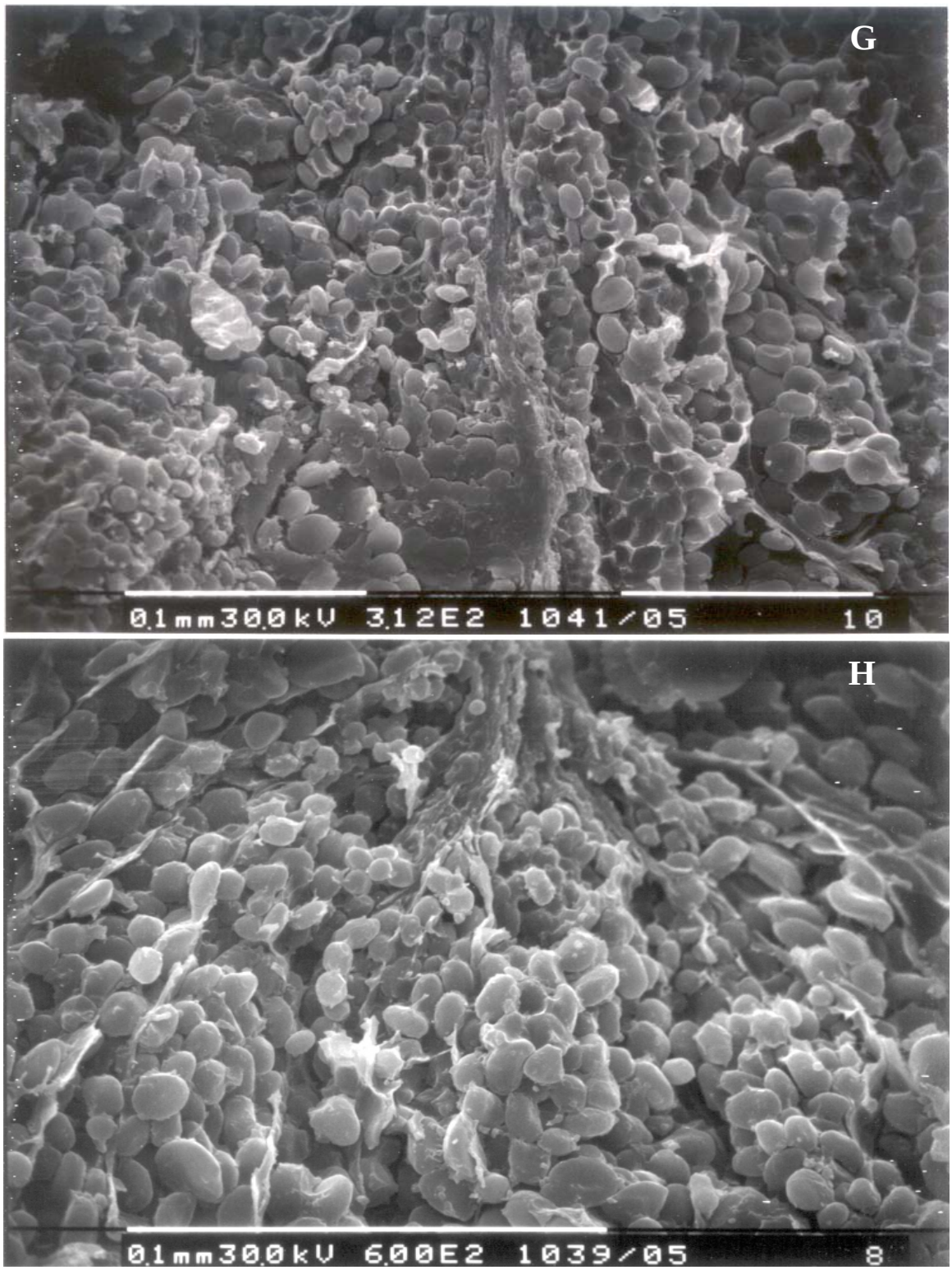

Figure 3.8. Scanning Electron Micrographs of Valier (G) and TR253 (H) central endosperm illustrating the starch-protein matrix of the genotypes as grown at Wakaw in 2004.

Table 3.3. Valier and TR253 *in situ* dry matter disappearance characteristics (factorial analysis), grown at Brandon, Saskatoon (KCRF and SF) and Wakaw sites in 2004.

Factor	Subfactor		Trait					
			Kd	Fraction (% DM)			EDDM	RUDM
			(%/hr)	A	B	C	(%)	(%)
Genotype	Valier		11.4	8.5	62.1 $_a$	29.4	48.8	51.2
	TR253		14.3	8.9	56.5 $_b$	34.6	47.9	52.1
	SE		0.93	0.19	1.66	1.64	0.56	0.56
Environment	Brandon		11.4	8.9	58.0	33.2	46.4	53.6
	Saskatoon	KCRF	13.9	8.2	60.0	31.8	50.0	50.0
		SF	14.3	9.4	58.9	31.8	49.4	50.6
	Wakaw		12.1	8.5	61.1	30.4	47.7	52.3
	SE		1.32	0.27	2.35	2.32	0.79	0.79
Pr>F	Genotype		0.23	0.37	0.04	0.06	0.21	0.21
	Environment		0.68	0.27	0.62	0.80	0.06	0.06
	G*E		0.15	0.19	0.69	0.57	0.65	0.65

Within genotype or environment, values in the same column followed by the same subscript do not differ (P<0.05).

3.3.3 *In situ* Rumen Degradation Results

Factorial analysis of variance of genotype x site revealed no significant differences between genotypes for Kd, A, C, EDDM or RUDM (P>0.21) (Table 3.3). However, significant differences were detected between genotypes for the B fraction (P<0.04). Valier had a greater potentially degradable fraction than TR253 (62.1% versus 56.5%, respectively). No location differences nor genotype x site interactions (P>0.06) were detected for any trait (Table 3.3).

Individual site examination revealed significant differences between genotypes not identified through factorial evaluation (Table 3.4). Kd differed significantly between genotypes at Brandon (P<0.01) and Saskatoon (SF) (P<0.08) but not at Saskatoon (KCRF) (P=0.18) or Wakaw (P=0.99) (Table 3.4). Kd was lower for Valier than TR253 at Brandon (9.0% versus 13.9%, respectively) and Saskatoon (SF) (10.9% versus 17.8%, respectively). Valier (7.49%) had significantly less A-fraction than TR253 (8.83%) at Saskatoon (KCRF) (P<0.03) but not at Brandon, Saskatoon (SF) or Wakaw (P>0.33) (Table 3.4). No differences in B, C, EDDM or RUDM were detected between genotypes at any site (P>0.13).

Table 3.4. Valier and TR253 *in situ* dry matter disappearance characteristics (individual site analysis), grown at Brandon, Saskatoon (KCRF and SF) and Wakaw sites in 2004.

Site	Genotype	Kd (%/hr)	A fraction (% DM)	B fraction (% DM)	C fraction (% DM)	EDDM (%)	RUDM (%)
Brandon	Valier	9.0 $_a$	8.7	62.1	29.2	46.6	53.4
	TR253	13.9 $_b$	9.0	53.8	37.1	46.2	53.8
	Mean	11.4	8.9	58.0	33.2	46.4	53.6
	SE	0.8	0.3	7.9	8.0	1.2	1.2
	Pr > t	0.009	0.33	0.13	0.15	0.77	0.77
Saskatoon (KCRF)	Valier	14.2	7.5 $_a$	60.6	31.9	50.1	49.9
	TR253	13.5	8.8 $_b$	59.4	31.8	49.9	50.1
	Mean	13.9	8.2	60.0	31.8	50.0	50.0
	SE	0.4	0.3	1.8	1.9	1.4	1.4
	Pr > t	0.18	0.03	0.53	0.97	0.91	0.91
Saskatoon (SF)	Valier	10.9 $_a$	9.3	63.1	27.7	49.7	50.3
	TR253	17.8 $_b$	9.5	54.7	35.9	49.2	50.8
	Mean	14.3	9.4	58.9	31.8	49.4	50.6
	SE	2.6	0.5	4.9	4.6	1.4	1.4
	Pr > t	0.08	0.72	0.19	0.17	0.74	0.74
Wakaw	Valier	12.2	8.6	64.1	27.2	49.0	51.0
	TR253	12.1	8.3	58.2	33.6	46.3	53.7
	Mean	12.1	8.5	61.1	30.4	47.7	52.3
	SE	3.8	0.9	9.5	9.2	1.5	1.5
	Pr > t	0.99	0.70	0.57	0.54	0.16	0.16

Within a site, values in the same column followed by the same subscript do not differ (P<0.10).

3.4 Discussion

As expected, since locations were selected for differences in SKCS hardness between the two genotypes, SKCS hardness results showed Valier as harder than TR253. Though differing in hardness, Valier and TR253 protein levels were similar. This is in agreement with Henry and Cowe (1990) who reported that barley grain hardness was independent of protein content. Valier and TR253 feed particle size was also similar, contrary to the relationship between grain hardness and particle size in wheat, where softer wheat produces flour with a smaller particle size (AACC, 1985). Feed particle size measurement after coarse rolling may not be sensitive enough to detect the subtle differences in grain hardness identified by the SKCS. The SKCS hardness calibration is based on percentage of flour particles remaining above a 155 and 470-μm sieve (Gaines et al., 1996). In this study, feed particle size was measured on considerably larger sieves, ranging from 1000 to 3350-μm.

Valier and TR253 SEM endosperm observations showed ultrastructure differences between Valier and TR253. At three of four sites, Valier was observed to have more starch-associated protein and a compact structure in central endosperm regions versus TR253, which had a mealier structure with limited starch-associated protein. This is in agreement with Brennen et al. (1996) who reported that good malting barley (TR253) had limited protein-associated starch and softer/mealier grain.

Middle endosperm areas of Valier and TR253, however, were variable, with apparent environmental influences. At Brandon and Wakaw, Valier had extensive starch-protein association compared to TR253. However, at Saskatoon (KCRF) the opposite was noted with TR253 having more starch-protein association and at Saskatoon (SF) no differences were detected. Of note, Brandon had the lowest mean protein level of the four sites with both genotypes having low protein. Nonetheless, Valier from Brandon appeared to have the most starch-associated protein and intact protein sheets of all samples. This may be attributed to environmental conditions at Brandon during grain maturation. Stenvert and Kingswood (1977) reported that elevated temperatures during grain desiccation in wheat resulted in the coalescence of endosperm protein and the formation of a continuous starch-protein matrix. In contrast, Wallwork et al. (1998) reported that higher temperatures during grain filling in barley resulted in mealier endosperm. However, determining the environmental influences on starch-protein association was beyond the scope of this project.

Additionally, TR253 had more dislodged B-type starch granules compared to Valier. Both Palmer (1991) and Brennen et al. (1996) reported that "good" malting barley had a loose protein association with B-type granules compared to "poor" malting barley.

In situ examination of DMDR revealed conflicting results. Factorial analysis of genotype and site effects resulted in no difference in Kd between Valier and TR253 and no genotype by site interaction. However, analysis of individual sites showed Valier had significantly slower DMDR than TR253 at Brandon and Saskatoon (SF) but not at Saskatoon (KCRF) or Wakaw. High residual error may have masked genotypic differences when analyzed factorially. Yu et al. (2003) similarly compared Kd of Valier (10.4% h^{-1}) with the malt barley variety Harrington (19.2% h^{-1}) and surprisingly found no difference in Kd when analyzed as a factorial, examining variety and processing method.

In situ examination is understandably variable, influenced by animal and microbial interrelation, among animals over time or even within an animal on a given day (Vanzant et al., 1998). In this study, Kd of genotypes ranged from 9.0 to 17.8% h^{-1} across environments, similar to rates reported by Yu et al. (2003) (10.4 to 19.2% h^{-1}) and Ramsey et al. (2001) (6.0 to 16.0% h^{-1}) for rolled barley. To compare *in situ* experiment variability, Ramsey et al. (2001) calculated the ratio of standard error of the measurements to mean rate of disappearance (*in situ* variability ratio). In the current study, the calculated *in situ* variability ratio for the factorial analysis of genotypes was 0.07 h^{-1}. For individual site analysis, ratios were 0.07, 0.03, 0.18 and 0.31 h^{-1} for Brandon, Saskatoon (KCRF), Saskatoon (SF) and Wakaw, respectively. This variability is equal to, if not lower, than that reported by Boss and Bowman (1996b) (0.25 h^{-1} ground samples) and Ramsey et al. (2001) (0.13 and 0.18 h^{-1} ground and rolled samples, respectively), who reported no differences in Kd between genotypes. However, with an *in situ* variability ratio of 0.10 h^{-1}, Lehman et al. (1995) reported significant barley genotype differences. In the current study (factorial analysis), there was no genotypic difference in Kd although the calculated *in situ* variability ratio (0.07 h^{-1}) was lower than that reported by Lehman et al. (1995). However, individual site analysis revealed significant differences between Valier and TR253 at Brandon and Saskatoon (SF) but not at Saskatoon (KCRF), where the ratio was only 0.03 h^{-1}. This suggests that environment did influence Kd of these genotypes, contrary to Lehman et al. (1995) who found no genotype by location effect ($P>0.05$) when evaluating eight barley varieties.

Ultrastructural differences between Valier and TR253 endosperm did not explain the *in situ* results. At Brandon, the tight starch-protein matrix in Valier may have had retarded degradation through delayed rumen microbial access to starch granules. However at Saskatoon (SF), Valier was also slower degrading but without the remarkable starch-protein association identified at Brandon. At Saskatoon (KCRF), TR253 had more starch-associated protein than Valier but there was no difference in Kd. *In situ* variability was greater at Wakaw, which may have masked potential genotype differences.

The current study did not establish a clear connection between SKCS hardness and DMDR. Prior research by Ramsey et al. (2001) using eight diverse barley cultivars also did not establish a connection between grain hardness (grinding time) and DMDR. Conversely in wheat, Moss and Givens (2002) reported a relationship (R^2=0.396) between mealiness and rumen degradable starch (8 hours), while Swan et al. (2006) found that increased puroindoline levels (i.e. increased softness) in wheat slowed DMDR.

From the current study, it can be concluded that, while genotypic differences exist in grain hardness between Valier and TR253, these did not translate into consistent differences in DMDR. As a result, there was no logic to further examination of DMDR in the 245 lines of the Valier x TR253 DH population.

4. EVALUATION OF GENOTYPE AND ENVIRONMENT ON BARLEY GRAIN HARDNESS

4.1 Introduction

Grain hardness may reflect barley's suitability for feed and could be an important simple selection tool for plant breeders attempting to improve feed quality. Various methods for hardness measurement have been developed based on differing aspects of hardness, such as crushing force, particle size and endosperm compaction. While all aspects are related to hardness, genotypes may respond distinctively between methods. In selecting the most appropriate screening tool for hardness, a breeder must be cognizant of differences between methods and potential environmental and compositional influences. Environmental conditions during grain development may alter gene expression, influencing hardness and making selection difficult. Identifying genotype by environment interaction is important to recognizing potential selection success.

The objectives of this experiment were to:

1) evaluate genotypic response and environmental influence on grain hardness methodology;
2) evaluate environmental effect on genotypes and hardness;
3) evaluate the influence of beta-glucan and protein on hardness; and
4) determine differences in hardness between Valier and TR253.

4.2 Materials and Methods

4.2.1 Grain Sample Production

Eight two-row spring barley genotypes were grown in field trials at twelve Western Canadian sites during 2003 and 2004. The genotypes included six feed varieties (CDC Bold, CDC Dolly, CDC Helgason, CDC Trey, McLeod, and Valier), one malting variety (Newdale) and one malting breeding line (TR253).

Valier is a two-row feed variety, registered by the Montana Agricultural Experiment Station (Bozeman, USA) in 1999 for its good agronomic performance and improved cattle-

feeding characteristics. TR253 is a malting barley breeding line developed at the Agriculture and Agri-Food Canada (AAFC) Brandon Research Centre (Brandon, Canada). It combines good agronomic performance and malting quality.

In 2003, trials were grown at Brandon, Saskatoon (GD), Hamiota, Saskatoon (KCRF), Lacombe and Saskatoon (SF). Saskatoon sites differed in seeding date and soil type and were considered distinct from each other. In 2004, trials were grown at Brandon, Saskatoon (GD), Saskatoon (KCRF), Saskatoon (SF), Wakaw and Watrous. Grain was harvested after physiological maturity, air-dried to less than 14.5% moisture for proper storage and allowed to equilibrate before analysis. Entries were sieved to increase relative uniformity (Blum et al., 1960; Pomeranz et al., 1985; Gaines et al., 1996). Samples from 2003 were screened such that only seed passing through a 2.5 x 18.75-mm slotted sieve and remaining on a 2.3 x 18.75-mm slotted sieve were used. Samples from 2004 were screened such that only seed passing through a 3.1 x 18.75-mm slotted sieve and those remaining on a 2.9 x 18.75-mm slotted sieve were retained for further evaluation. Sieve sizes varied between years due to differences in growing conditions, with samples from 2003 being thinner than 2004.

4.2.2 Grain Analysis

4.2.2.1 SKCS Hardness, Seed Weight, Diameter and Moisture

SKCS hardness was determined as already described in Section 3.2.3.1. Three hundred whole seed per sample were evaluated using the SKCS 4100 (Perten Instruments, Springfield, IL) to determine SKCS seed weight, diameter and moisture.

4.2.2.2 Milling Energy

Five-gram samples were milled in a 'Comparamill' flourmill at the Scottish Research Institute in Invergowrie, Dundee, Scotland to determine milling energy (joules). Higher milling energy indicates greater hardness.

4.2.2.3 Light Reflectance

Fifty seed per sample were pearled to 85% initial weight (i.e., to remove hull) and sliced longitudinally, with one seed half evaluated. Two incandescent lights were placed adjacent to a Leica MZFLIII light microscope, equipped with a Q-Imaging Micropublisher 3.3 RTV camera.

Seed halves were secured in plastercine with the exposed endosperm facing upward. Each illuminated endosperm was photographed (exposure 2.2 milliseconds, gain 50%, offset 0%, ROI sampling 2x2, size 1024x768 pixels) using Empix Imaging's Northern Eclipse V7.0™ microscope image acquisition and analysis software. Digital images were converted to inverted grayscale and analyzed for endosperm reflected light intensity (1000 light pixels per mm^2) using Bio-Rad's Quantity One® 1-D analysis software.

4.2.2.4 Feed Particle Size Analysis

Refer to 3.2.3.3. In addition, Large particle size (% Large Particles) was calculated by the percentage of material by weight remaining above 2.36-mm sieve. Mid-size particle size (% Mid-size Particles) was calculated by the percent of material remaining above a 1.40-mm sieve and passing through a 2.36-mm sieve. Fine particle size (% Fine Particles) was calculated by the percentage of material that passed through a 1.40-mm sieve.

4.2.2.5 Protein

Refer to 3.2.3.2.

4.2.2.6 Beta-glucan

Ten-gram samples were ground using a Udy cyclone mill. Twenty-five milligrams of ground barley was analyzed for beta-glucan content by flow injection analysis (Aastrup, 1988) using the Eppendorf Flow Injection Analyzer.

4.2.3 Statistical Analysis

To test differences between 8 genotypes and 2 environments (years), analysis of variance was conducted for all traits using PROC GLM (SAS Institute, Cary, NC, USA, 2006). Growing sites were considered replicates. Differences among genotypes were tested using Tukey's studentized range test (significance level $P<0.05$). Correlations and relationships between hardness, protein, beta-glucan and feed particle size of genotype and site means were determined by PROC CORR and PROC REG, respectively.

4.3 Results

Analysis of variance revealed significant differences between genotypes and environments for all traits measured ($P<0.001$) except for SKCS diameter and moisture, which showed environment ($P<0.0001$) but no genotype differences ($P>0.45$) (Table 4.1). No genotype by environment interaction was detected for milling energy, light reflectance, protein, or SKCS weight ($P>0.10$). Non-cross over genotype by environment interaction was detected for SKCS hardness ($P<0.004$) and beta-glucan ($P<0.0001$), as the magnitude of genotype differences varied between years, but ranking did not. All environments were combined for final analysis of grain hardness, protein, and beta-glucan.

SKCS hardness of genotypes ranged from 38.2 to 56.1 (Table 4.1). McLeod was hardest, followed by Valier and CDC Dolly. CDC Trey, Newdale, TR253, CDC Helgason, and CDC Bold followed, with CDC Bold the softest. SKCS hardness ranged from 35.2 to 55.8 across environments (data not shown). Milling energy of genotypes ranged from 617 to 736 joules (Table 4.1). McLeod and CDC Dolly required significantly more energy to mill, followed by Valier, Newdale, CDC Helgason, CDC Trey and TR253. CDC Bold required the least energy to mill, indicating a softer endosperm. Milling energy ranged from 629 to 701 joules across environments (data not shown). Light reflectance ranged from 19.2 to 23.8 thousand pixels/mm^2 across genotypes (Table 4.1). McLeod was hardest (steeliest), with Valier, CDC Dolly and CDC Trey following. CDC Helgason, Newdale and TR253 were softer, with CDC Bold being the softest (mealiest). Light reflectance ranged from 20.0 to 24.5 thousand pixels/mm^2 across environments (data not shown).

Protein content of genotypes ranged from 11.1 to 12.3% (Table 4.1). McLeod, CDC Dolly, Valier, Newdale and CDC Helgason contained more protein followed by TR253, CDC Bold and CDC Trey. Protein content varied from 8.9% to 15.5% across environments (data not shown). Percent beta-glucan of genotypes ranged from 3.4% to 4.7% (Table 4.1). CDC Dolly had the highest beta-glucan followed by Valier, Newdale, TR253 and McLeod. CDC Helgason, CDC Trey and CDC Bold followed, with CDC Bold having the lowest levels. Beta-glucan varied from 3.8% to 4.3% across environments (data not shown).

Table 4.1. Mean grain hardness, protein, beta-glucan, SKCS seed weight, diameter and moisture of eight barley genotypes as grown at twelve Western Canadian sites, 2003 and 2004.

Genotype	SKCS Hardness	Milling Energy (j)	Light Reflectance (1000 pixels/mm^2)	Protein (%)	Beta-glucan (%)	SKCS Seed		
						Weight (g)	Diameter (mm)	Moisture (%)
McLeod	56.1 $_{a}$	736 $_{a}$	19.2 $_{a}$	12.3 $_{a}$	3.98 $_{cd}$	51.6 $_{ab}$	2.62	10.3
Valier	50.4 $_{b}$	691 $_{b}$	21.1 $_{b}$	11.9 $_{ab}$	4.35 $_{b}$	48.0 $_{c}$	2.57	10.2
CDC Dolly	48.4 $_{bc}$	733 $_{a}$	21.4 $_{bc}$	12.0 $_{ab}$	4.72 $_{a}$	51.5 $_{ab}$	2.64	10.3
CDC Trey	47.1 $_{cd}$	645 $_{d}$	21.6 $_{bc}$	11.1 $_{c}$	3.88 $_{d}$	50.4 $_{b}$	2.58	10.3
Newdale	45.4 $_{cd}$	675 $_{bc}$	22.5 $_{cde}$	11.9 $_{ab}$	4.12 $_{c}$	50.3 $_{b}$	2.57	10.4
TR253	44.8 $_{d}$	644 $_{d}$	23.6 $_{de}$	11.5 $_{bc}$	4.01 $_{cd}$	48.5 $_{c}$	2.59	10.1
CDC Helgason	44.4 $_{d}$	663 $_{cd}$	22.3 $_{bcd}$	11.8 $_{abc}$	3.88 $_{d}$	48.3 $_{c}$	2.52	10.1
CDC Bold	38.2 $_{e}$	617 $_{e}$	23.8 $_{e}$	11.4 $_{bc}$	3.40 $_{e}$	52.8 $_{a}$	2.54	10.4
Mean	46.9	676	21.9	11.7	4.04	50.2	2.58	10.2
S.E.	0.7	5.3	0.3	0.2	0.04	0.4	0.01	0.1
Pr > F	0.001	<0.0001	<0.0001	<0.0001	<0.001	<0.0002	0.56	0.45

Values in the same column followed by the same subscript do not differ (P<0.05).

Table 4.2. Feed particle size analysis of eight barley cultivars as grown at twelve Western Canadian sites (2003 and 2004), processing methods combined.

	% Particles > sieve size (mm)					
Genotype	3.35	2.36	2.00	1.40	1.00	<1.00
Newdale	15.6 $_{a}$	38.7 $_{bc}$	19.1 $_{d}$	17.2 $_{abc}$	4.62 $_{bc}$	4.86 $_{cd}$
McLeod	14.9 $_{ab}$	39.5 $_{abc}$	19.5 $_{cd}$	16.4 $_{cd}$	4.52 $_{c}$	5.16 $_{bc}$
CDC Dolly	14.0 $_{abc}$	41.2 $_{a}$	19.8 $_{bcd}$	16.0 $_{d}$	4.22 $_{d}$	4.82 $_{d}$
CDC Helgason	13.2 $_{bcd}$	39.5 $_{abc}$	20.7 $_{bc}$	17.1 $_{abc}$	4.58 $_{bc}$	4.91 $_{cd}$
CDC Trey	12.5 $_{cd}$	39.5 $_{abc}$	20.2 $_{bcd}$	17.4 $_{abc}$	4.98 $_{a}$	5.40 $_{ab}$
TR253	12.1 $_{cd}$	38.9 $_{bc}$	21.0 $_{ab}$	18.1 $_{ab}$	4.85 $_{ab}$	5.03 $_{cd}$
Valier	12.1 $_{cd}$	40.7 $_{abc}$	20.7 $_{bc}$	17.1 $_{bc}$	4.59 $_{bc}$	4.87 $_{cd}$
CDC Bold	11.5 $_{d}$	37.6 $_{c}$	22.1 $_{a}$	18.2 $_{a}$	5.11 $_{a}$	5.64 $_{a}$
Mean	13.2	39.5	20.4	17.2	4.69	5.09
S.E.	0.5	0.5	0.3	0.2	0.07	0.07
Pr > F	0.002	0.01	0.02	0.02	0.01	0.02

Values in the same column followed by the same subscript do not differ (P<0.05).

SKCS seed weight of genotypes ranged from 48.0 to 52.8 grams (Table 4.1). CDC Bold, McLeod and CDC Dolly had the largest seed, followed by CDC Trey and Newdale. TR253, CDC Helgason and Valier had the smallest seeds. SKCS seed weight varied from 44.0 to 56.2 grams across environments (data not shown). No difference between genotypes was detected for SKCS diameter or moisture.

Analysis of variance of feed particle size revealed significant differences between processing methods (P<0.05) for all measurements, except for particle size 3.35-mm and mean particle size (P>0.07). However, particle size 3.35-mm and mean particle size showed an interaction between genotype and processing (P<0.01), although generally non-cross over. Year by processing interaction was detected for particle size 3.35, 2.36, and 1.40-mm, large and mid-sized particles, and mean particle size (P<0.003), although again generally non-cross over. All processing methods and environments were combined for final feed particle size analysis (Table 4.2).

Particle size 3.35-mm of genotypes ranged from 11.5 to 15.6% (Table 4.2). Newdale, McLeod, and CDC Dolly had the highest percentage, followed by CDC Helgason, CDC Trey, TR253 and Valier. CDC Bold had the lowest percentage 3.35-mm particles. Particle size 3.35-mm varied from 10.5 to 19.5% across environments (data not shown).

Particle size 2.36-mm ranged from 37.6 to 41.2%, with CDC Dolly, Valier, McLeod, CDC Helgason, and CDC Trey having the largest percentage (Table 4.2). TR253, Newdale and

CDC Bold followed with CDC Bold having the lowest. Particle size 2.36-mm varied from 37.7 to 43.5% across environments (data not shown).

Particle size 2.00-mm of genotypes ranged from 19.1 to 22.1% (Table 4.2). CDC Bold and TR253 had the greatest portion of 2.00-mm particles with CDC Helgason, Valier, CDC Trey and CDC Dolly following. McLeod and Newdale had the lowest percentage of this fraction. Environments varied from 18.4 to 22.2% in 2.00-mm particle size (data not shown).

Particle size 1.40-mm ranged from 16.0 to 18.2%, with CDC Bold, TR253, CDC Trey, Newdale and CDC Helgason having the largest percentages (Table 4.2). Valier, McLeod and CDC Dolly had the lowest portion of this fraction. Particle size 1.40-mm varied from 14.8 to 18.7% across environments (data not shown).

Particle size 1.00-mm of genotypes ranged from 4.2 to 5.1% (Table 4.2). CDC Bold, CDC Trey and TR253 had the largest proportion of this size, followed by Newdale, Valier, CDC Helgason, McLeod and CDC Dolly. CDC Dolly had the lowest proportion of 1.00-mm particles. Environments varied from 3.9 to 5.1% for this particle size (data not shown).

Particle size <1.00-mm of genotypes ranged from 4.8 to 5.6% (Table 4.2). CDC Bold and CDC Trey had the largest portion of this fraction, followed by McLeod, TR253, CDC Helgason, Valier, Newdale, and CDC Dolly. CDC Dolly had the lowest portion of particles <1.00-mm. Environments varied from 3.9 to 5.9% for particles <1.00-mm (data not shown).

Mean particle size of genotypes ranged from 2307 to 2465-µm (Table 4.3). Newdale, McLeod, CDC Dolly and CDC Helgason had the largest mean particle size, with Valier, CDC Trey, TR253 and CDC Bold following. CDC Bold had the smallest mean particle size. Mean particle size varied from 2290 to 2645-µm across environments (data not shown).

Percent large particles (>2.36-mm) of genotypes varied from 49.0 to 55.2%, with CDC Dolly, McLeod and Newdale having the largest percentages (Table 4.3). Valier, CDC Helgason, CDC Trey, TR253 and CDC Bold followed, with CDC Bold having the lowest percent large particles. Percent large particles (>2.36-mm) ranged from 49.3 to 57.9% across environments (data not shown).

Genotype percent mid-sized particles (1.40-2.36-mm) ranged from 35.7 to 40.2% (Table 4.3). CDC Bold and TR253 had the largest percentage, followed by CDC Helgason, Valier, CDC Trey, Newdale, McLeod with CDC Dolly having the lowest percentage. Mid-sized particles varied from 35.1 to 40.6% across environments (data not shown).

Table 4.3. Mean, large, mid-size, and fine feed particle size of eight barley genotypes as grown at twelve Western Canadian sites (2003 and 2004), processing methods combined.

Genotype	Mean Particle Size (μm)	% Particles - sieve sizes (mm)		
		% Large (>2.36)	% Mid-sized (1.40-2.36)	% Fine (<1.40)
Newdale	2465 $_{a}$	54.3 $_{ab}$	36.2 $_{cde}$	9.48 $_{cd}$
McLeod	2457 $_{ab}$	54.4 $_{ab}$	35.9 $_{de}$	9.68 $_{c}$
CDC Dolly	2450 $_{ab}$	55.2 $_{a}$	35.7 $_{e}$	9.03 $_{d}$
CDC Helgason	2403 $_{abc}$	52.7 $_{bc}$	37.8 $_{bc}$	9.49 $_{cd}$
Valier	2379 $_{bcd}$	52.8 $_{bc}$	37.7 $_{bc}$	9.47 $_{cd}$
CDC Trey	2358 $_{cd}$	52.0 $_{c}$	37.6 $_{bcd}$	10.39 $_{ab}$
TR253	2350 $_{cd}$	51.1 $_{cd}$	39.0 $_{ab}$	9.88 $_{bc}$
CDC Bold	2307 $_{d}$	49.0 $_{d}$	40.2 $_{a}$	10.75 $_{a}$
Mean	2396	52.7	37.5	9.77
S.E.	20	0.5	0.4	0.13
Pr > F	0.002	0.003	0.005	0.01

Values in the same column followed by the same subscript do not differ (P<0.05).

Genotype percent fine-particles (<1.40-mm) ranged from 9.0 to 10.8% (Table 4.3). CDC Bold and CDC Trey had the most fine particles. TR253, McLeod, CDC Helgason, Newdale, Valier followed. CDC Dolly had the least fine particles. Fine particles (<1.40-mm) varied from 7.8 to 11.0 across environments (data not shown).

Pearson's correlation analysis is summarized in Table 4.4. Milling energy was positively related (n=8) to SKCS hardness (R^2=0.70, P<0.009) (Figure 4.1), protein (R^2=0.74, P<0.006) (Figure 4.2), and beta-glucan (R^2=0.56, P<0.03) (Figure 4.3). However, the milling energy versus beta-glucan correlation was non-significant (P=0.57, n=6) when high (CDC Dolly) and low (CDC Bold) beta-glucan genotypes were removed from the analysis. In contrast, the milling energy versus beta-glucan correlation was stronger (r = 0.96, P<0.0005, n=7) when the high protein genotype McLeod was removed. Effects of the small genotype sample size were evident throughout the study for some trait interrelationships.

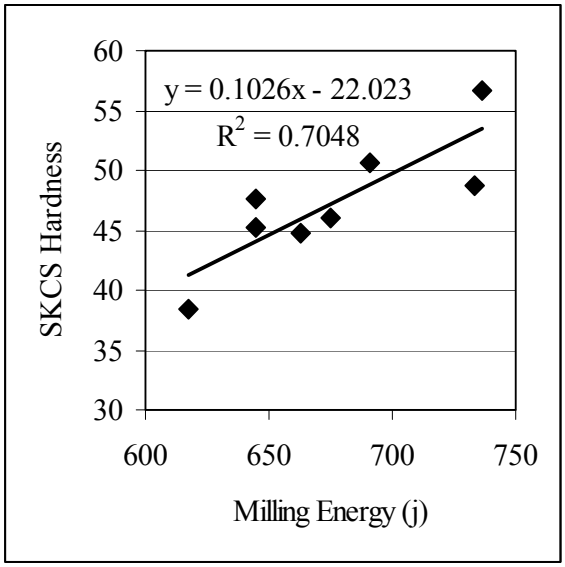

Figure 4.1. Relationship between SKCS hardness and milling energy for eight barley genotypes.

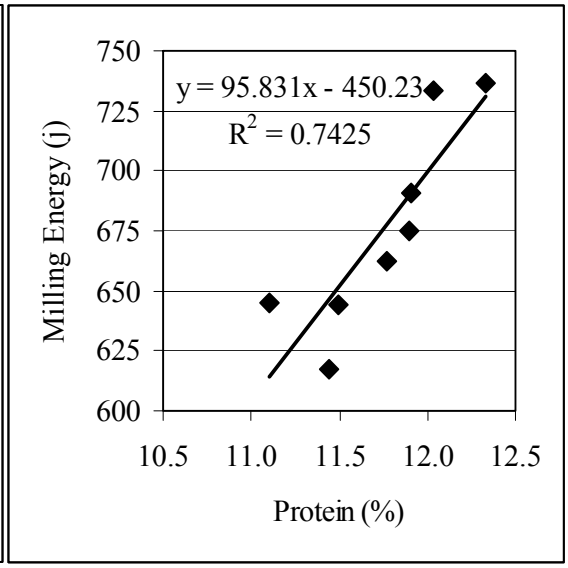

Figure 4.2. Relationship between milling energy and protein for eight barley genotypes.

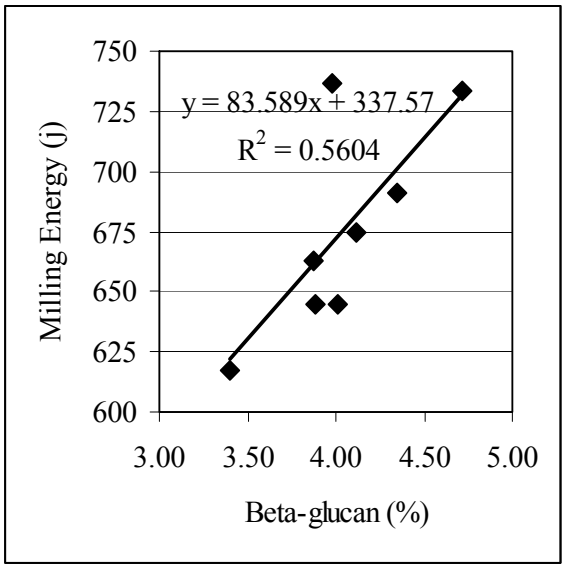

Figure 4.3. Relationship between milling energy and beta-glucan for eight barley genotypes.

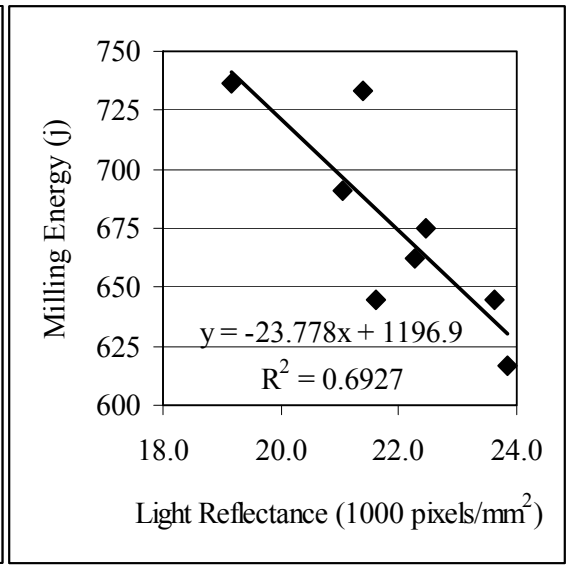

Figure 4.4. Relationship between milling energy and light reflectance for eight barley genotypes.

Table 4.4. Pearson correlations between grain hardness, protein, beta-glucan, mean particle size, and large, mid-sized and fine particles (N=8).

	Milling Energy	SKCS Hardness	Light Reflect	Protein (%)	Beta-glucan (%)	SKCS Weight	MPS (All RG)	MPS (1.3-mm RG)	MPS (1.7-mm RG)	% Particles (All RG)		
										Large	Mid-sized	Fine
Milling Energy (j)	1											
SKCS Hardness	0.84	1										
	P<0.009											
Light Reflectance	-0.83	-0.94	1									
	P<0.01	*P<0.0005*										
Protein (%)	0.86	0.65	-0.65	1								
	P<0.006	*P=0.08*	*P=0.08*									
Beta-glucan (%)	0.75	0.54	-0.43	0.50	1							
	P<0.03	*P=0.17*	*P=0.29*	*P=0.20*								
SKCS Weight (g)	0.09	-0.11	-0.07	0.08	-0.25	1						
	P=0.83	*P=0.80*	*P=0.87*	*P=0.86*	*P=0.55*							
MPS (All RG)	0.83	0.65	-0.63	0.78	0.63	0.05	1					
	P<0.01	*P=0.08*	*P=0.09*	*P<0.02*	*P=0.10*	*P=0.91*						
MPS (1.3-mm RG)	0.88	0.69	-0.65	0.69	0.85	-0.04	0.93	1				
	P<0.004	*P=0.06*	*P=0.08*	*P=0.06*	*P<0.007*	*P=0.91*	*0.0008*					
MPS (1.7-mm RG)	0.77	0.61	-0.60	0.79	0.52	0.08	0.99	0.86	1			
	P<0.02	*P=0.11*	*P=0.12*	*P<0.02*	*P=0.19*	*P=0.85*	*<0.0001*	*P<0.005*				
Large Particles	0.89	0.73	-0.70	0.72	0.80	-0.04	0.96	0.99	0.91	1		
	P<0.003	*P<0.04*	*P=0.05*	*P<0.04*	*P<0.02*	*P=0.92*	*P<0.0002*	*P<0.0001*	*P<0.002*			
Mid-sized Particles	-0.87	-0.75	0.75	-0.67	-0.72	-0.07	-0.95	-0.97	-0.91	-0.98	1	
	P<0.005	*P<0.03*	*P<0.03*	*P=0.07*	*P<0.05*	*P=0.86*	*P<0.0004*	*P<0.0001*	*P<0.002*	*P<0.0001*		
Fine Particles	-0.77	-0.52	0.44	-0.72	-0.87	0.37	-0.79	-0.87	-0.74	-0.86	-0.75	1
	P<0.02	*P=0.18*	*P=0.28*	*P<0.04*	*P<0.005*	*P=0.37*	*P<0.02*	*P<0.004*	*P<0.04*	*P<0.007*	*P<0.03*	

MPS = mean particle size
1.3-mm RG = roller gap size 1.3-mm (over-processed)
1.7-mm RG = roller gap size 1.7-mm (minimally processed)
All RG = combined means of processing methods

Both milling energy (R^2=0.69, P<0.01) (Figure 4.4) and SKCS hardness (R^2=0.88, P<0.0005) (Figure 4.5) were negatively related to light reflectance. SKCS hardness and protein (P=0.08), SKCS hardness and beta-glucan (P=0.17), light reflectance and protein (P=0.08) and light reflectance and beta-glucan (P=0.29) were not significantly correlated. However, the SKCS hardness versus protein correlation was significant (r = 0.80, P<0.05, n=6) when high (McLeod) and low (CDC Trey) protein genotypes were removed from the analysis. Similar results were found for light reflectance and protein (r = -0.92, P<0.0009, n=6).

Mean particle size from combined processing methods (ALL RG) was positively related to milling energy (R^2=0.68, P<0.01) (Figure 4.6) and protein (R^2=0.61, P<0.02) (Figure 4.7). Separate analysis of processing methods showed mean particle size of over-processed material (1.3-mm RG) positively correlated with beta-glucan (r = 0.85, P<0.007). In contrast, mean particle size of minimally processed material (1.7-mm RG) was positively correlated with protein (r = 0.79, P<0.02) rather than beta-glucan (P=0.19). However, mean particle size and protein/beta-glucan correlations were non-significant (P=0.08, n=6) when high (CDC Dolly) and low (CDC Bold) beta-glucan genotypes were removed from the analysis. In contrast, mean particle size and protein/beta-glucan correlations became more significant when high (McLeod) and low (CDC Trey) protein genotypes were removed from the analysis. Mean particle size

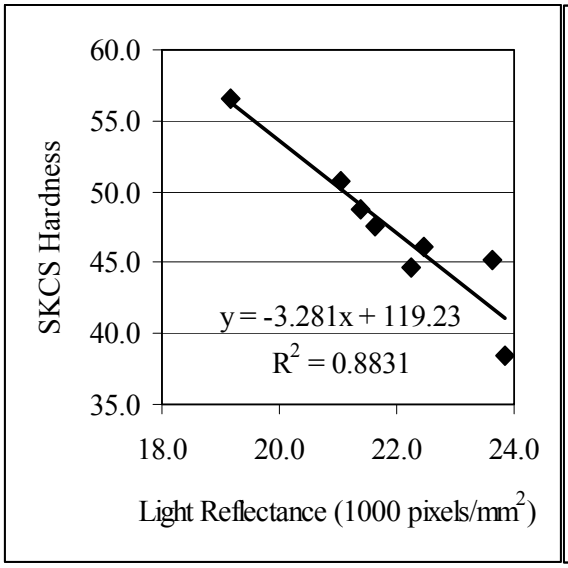

Figure 4.5. Relationship between SKCS hardness and light reflectance for eight barley genotypes.

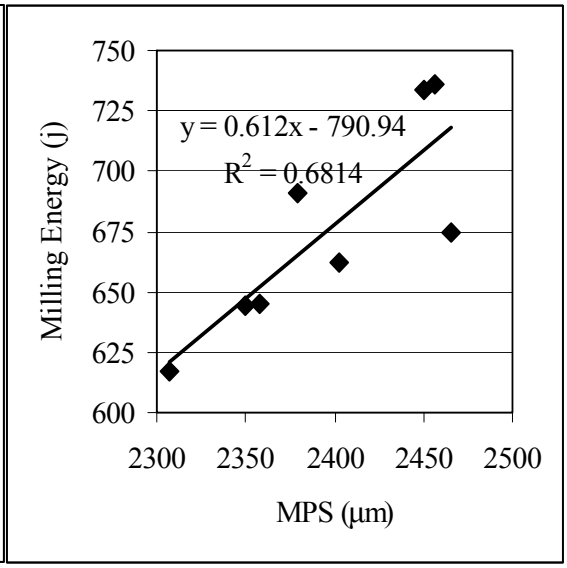

Figure 4.6. Relationship between milling energy and mean particle size (ALL RG) for eight barley genotypes.

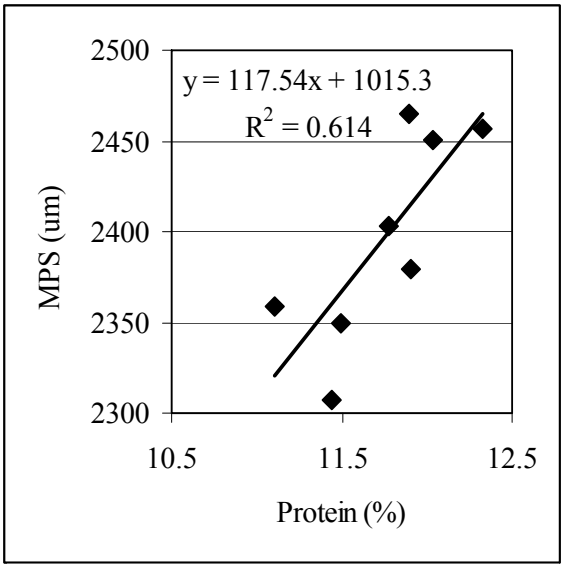

Figure 4.7. Relationship between mean particle size (ALL RG) and protein for eight barley genotypes.

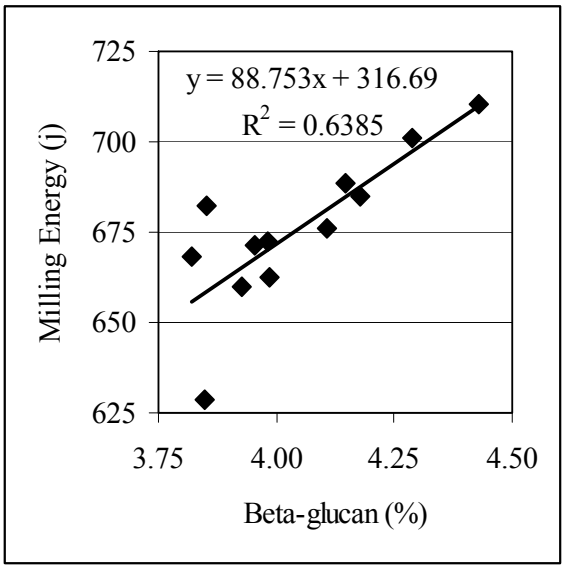

Figure 4.8. Relationship between milling energy (j) and beta-glucan across twelve environments.

(over-processed) was positively correlated with protein ($r = 0.93$, $P<0.007$, $n=6$) and beta-glucan ($r = 0.90$, $P<0.01$, $n=6$). Mean particle size (minimally processed) remained positively correlated with protein ($r = 0.81$, $P<0.05$, $n=6$) but not beta-glucan ($P=0.20$).

From combined processing methods (ALL RG), percent large particles was positively correlated with milling energy ($r = 0.89$, $P<0.003$), SKCS hardness ($r = 0.73$, $P<0.04$), protein ($r = 0.72$, $P<0.04$) and beta-glucan ($r = 0.80$, $P<0.02$). Percent mid-sized particles was negatively correlated with milling energy ($r = -0.87$, $P<0.005$), SKCS hardness ($r = -0.75$, $P<0.03$), and beta-glucan ($r = -0.72$, $P<0.05$) while positively correlated with light reflectance ($r = 0.75$, $P<0.03$). Percent fine particles was negatively correlated with milling energy ($r = -0.77$, $P<0.02$), protein ($r = -0.72$, $P<0.04$) and beta-glucan ($r = -0.87$, $P<0.005$) only.

To evaluate environmental influences on grain hardness, Pearson's correlation analysis was performed on protein, beta-glucan and hardness site means. Milling energy, SKCS hardness, light reflectance and mean particle size (ALL RG) were not correlated with protein levels across the twelve environments ($P>0.25$). Similarly, SKCS hardness and mean particle size (ALL RG) were not correlated with beta-glucan levels ($P>0.05$, $n=12$). However, milling energy was positively related to beta-glucan levels ($R^2=0.64$, $P<0.002$) (Figure 4.8) across the

twelve environments, while light reflectance/beta-glucan showed a negative trend ($r = -0.57$, $P=0.05$, $n=12$).

4.4 Discussion

Genotypes differed in SKCS hardness, milling energy and endosperm light reflectance with McLeod, CDC Dolly and Valier being consistently harder and CDC Bold softest. Hardness ranking of genotypes based on feed particle size differed slightly with Newdale, McLeod and CDC Dolly having larger particle size and CDC Bold small. Hardness methodology ranked genotypes similarly across environments, although levels varied at each. Stenvert and Kingswood (1977) reported similar findings for wheat and described that the genetic influence of hardness was modified by the environment without altering ranking. The exception in the current study was Valier and TR253 as large differences between these genotypes in 2003 were minimal in 2004 (data not shown).

Although SKCS hardness, milling energy and endosperm light reflectance methods correlated well, only milling energy showed a consistent relationship with feed particle size. Both milling energy and feed particle size measurements are products of shearing action from roller milling, whereas SKCS hardness is based on compression force. Muhamad and Campbell (2004) reported for wheat that the crushed grain after SKCS analysis contained larger particles compared to roller milling. Their findings may help explain the current results in which SKCS hardness (and light reflectance) was correlated with large and mid-sized particles but not fine particles or mean particle size. Light reflectance may also relate more to compression rather than shearing.

Milling energy appeared to be influenced by the protein and beta-glucan of genotypes, with harder grain having greater levels of both. These results are similar to Allison et al. (1979a), who found a positive correlation with milling energy and grain nitrogen (protein), and Henry and Cowe (1990) for milling energy and beta-glucan. However, the current finding is in contrast with Allison et al. (1979b) who found no correlation between milling energy and beta-glucan.

SKCS hardness and light reflectance were not correlated with protein or beta-glucan. Washington et al. (2001) also reported no correlation between protein and SKCS hardness but did find a significant correlation for SKCS hardness and beta-glucan. Chandra et al. (1997)

reported steeliness associated with higher levels of protein and beta-glucan. In the current study, light reflectance was measured using single transverse sections of multiple grains, whereas protein and beta-glucan were analyzed on blended whole grain samples. Therefore, protein and/or beta-glucan could be influencing light reflectance in the observed section without correlation to the whole grain measurement. Chandra et al. (1997) reported that mealy barley genotypes stored extra protein in the embryo rather than the central endosperm, as did steely barley.

Feed particle size may also be influenced by protein and beta-glucan content. Higher protein genotypes tended to remain in larger pieces with minimal processing compared to lower protein genotypes. This relationship was not evident with over-processed barley, where conversely beta-glucan influenced particle size. A plausible explanation is that with minimal roller force, an increased level of starch-associated protein maintained cohesiveness of the endosperm cellular structure, producing larger particles. However, an increased force caused the starch-protein matrix to shear, relying on the beta-glucan in the cell walls to maintain endosperm integrity.

Findings by Surber et al. (2000) suggest that increased mean particle size of barley reduced DMDR in cattle. In the current study, larger mean particle size was associated with increased grain protein and/or beta-glucan. Breeding for higher protein and beta-glucan may increase mean particle size of barley and indirectly reduce DMDR in cattle.

Milling energy may be a useful tool to identify barley with increased mean particle size. Milling energy is a quick method of evaluation that requires only one minute per sample and five grams of seed (Allison et al., 1979a). It would also allow earlier generation selection for this trait. Feed particle size analysis requires significantly more seed (50-grams) and time, with rolling and particle separation a two-step process.

Past findings by Stenvert and Kingswood (1977) showed that increased grain hardness was associated with increased protein levels over sites. Contrarily in the current study, hardness was not correlated with site protein levels. However, beta-glucan levels at each site did positively influence milling energy in a linear fashion. No such relationship was found with any other hardness measurement. The effect of environment on beta-glucan has been researched by Hesselman and Thomke (1982). They reported that beta-glucan formation is influenced by

temperature and rainfall during grain development with elevated temperatures and drier conditions before harvest resulting in higher beta-glucan levels.

In the current study, Valier and TR253 were significantly different for SKCS hardness, milling energy, light reflectance and beta-glucan. However, there were no differences in feed particle size. It was concluded that sufficient differences existed in grain hardness to evaluate Valier x TR253 DH population for SKCS hardness, milling energy and endosperm light reflectance but not feed particle size.

5. GRAIN HARDNESS INHERITANCE

5.1 Introduction

Heritability is defined as "the proportion of the observed variation in a progeny that is inherited" (Poehlman and Sleper, 1995). It gauges the usefulness of trait selection in populations, through measurement of genetic versus environmental variability. Improvements may be extremely limited in populations that are variable mainly due to environmental influences (low heritability), whereas quick advancements may be possible with highly heritable traits.

Heritability is calculated either in the broad or narrow-sense (Poehlman and Sleper, 1995). Broad-sense heritability is a measure of all genetic effects, including additive, dominant and epistasis, while narrow-sense heritability is a measure of only additive gene effects and is often more useful for plant breeding. By definition, a double haploid (DH) population has no dominance and imparts only additive and possibly epistatic gene action. Therefore, if no epistasis is present, broad and narrow-sense heritability will be equal. Choo and Reinbergs (1982) demonstrated that skewness and kurtosis coefficients may be used in determining epistasis in DH populations.

Limited research has been reported on grain hardness inheritance and heritability in barley. Allison (1986) showed hardness was heritable by tracing the mealiness of modern British malting barley cultivars to a few older mealy European parents. Thomas et al. (1996) and Fox et al. (2007b) reported high heritability in milling energy and SKCS hardness, respectively. No reports have been published on heritability of grain hardness as measured by light reflectance.

The objectives of this research were to:

1) examine the inheritance of grain hardness (SKCS hardness, milling energy, and light reflectance), beta-glucan and protein in a Valier x TR253 DH population;

2) estimate broad and narrow-sense heritability of hardness (SKCS hardness, milling energy, and light reflectance), beta-glucan and protein; and

3) examine the influence of beta-glucan and protein on hardness.

5.2 Materials and Methods

5.2.1 Valier and TR253

Valier was registered by the Montana Agricultural Experiment Station in 1999 for its good agronomic performance and improved cattle-feeding characteristics (Blake et al., 2002). Valier was developed from a cross between the malt barley Lewis and the feed barley Baronesse with selection for slower rate of dry matter disappearance compared to the parents. Calves fed Valier gained quicker than calves fed either Lewis or Baronesse (Boss et al., 1999).

TR253 is a malt barley breeding line developed at the Agriculture and Agri-Food Canada (AAFC) Brandon Research Centre (Brandon, Canada). It combines good agronomic performance with good malting quality.

5.2.2 Population Development

Two hundred and forty-five double haploid lines were developed at the University of Saskatchewan, Saskatoon, Canada in 2001 from F_1 plants using the *in vitro* microspore culture technique described by Kasha et al. (2001).

5.2.3 Grain Sample Production

Two hundred and forty-five double haploid lines were grown in hill-plot field trials at the University of Saskatchewan, Saskatoon, Canada in 2003 and 2004. The trial was designed as a modified RCBD, with two replications per year. Twelve (2003) and twenty (2004) repeated paired hill-plots of the parents Valier and TR253 were regularly interspersed throughout the trial. Grain was harvested after physiological maturity, air-dried to less than 14.5% moisture for proper storage and allowed to equilibrate before analysis. Samples were sieved to increase relative uniformity in size and shape for further analysis (Blum et al., 1960; Pomeranz et al., 1985; Gaines et al., 1996). Samples from 2003 were screened such that only seed passing through a 2.5 x 18.75-mm slotted sieve and remaining on a 2.3 x 18.75-mm slotted sieve were retained. Samples from 2004 were screened such that only seed passing through a 3.1 x 18.75-

mm slotted sieve and those remaining on a 2.9 x 18.75-mm slotted sieve were retained. Sieve sizes varied between years due to differences in growing conditions, with samples from 2003 being thinner than 2004.

5.2.4 Grain Analysis

5.2.4.1 SKCS Hardness, Seed Weight, Diameter and Moisture

Refer to 4.2.2.1.

5.2.4.2 Milling Energy

Refer to 4.2.2.2. Only the first replication of 2003 and 2004 material was analyzed.

5.2.4.3 Light Reflectance

Refer to 4.2.2.3. Thirty seed from one hundred corresponding lines were randomly chosen for analysis.

5.2.4.4 Protein

Refer to 3.2.3.2.

5.2.4.5 Beta-glucan

Refer to 4.2.2.6. Both replications from 2004 were analyzed.

5.2.5 Statistical Analysis

5.2.5.1 Parent and Population Characteristics

Parent trait differences were analyzed using PROC TTEST (for paired samples) (SAS Institute, Cary, NC, USA, 2006). Analysis of variance was measured for all population traits using PROC GLM. Population distribution characteristics were obtained using PROC UNIVARIATE, with normality of distributions determined by Shapiro-Wilk's (W) test for normality (significance level $P<0.05$). Transgressive segregation of the population was examined using parent standard deviations (significance level $P<0.05$) from individual years. Pearson's correlation analysis of genotype means was determined by PROC CORR, with relationships determined by PROC REG.

5.2.5.2 Heritability estimate

Broad-sense heritability (H^2) was estimated for SKCS hardness, light reflectance and protein using variance components obtained from PROC GLM (formula adapted from Comstock and Moll (1963)):

$$H^2 = \frac{V_G}{V_G + V_{GxY\,/\,Y} + V_{ERROR\,/\,YxR}}$$

Where V_G is genotypic variance, V_{GxY} is genotype by year interaction variance, and V_{ERROR} is residual error.

To test for epistasis, skewness (g_1) and kurtosis (g_2) coefficients (Choo and Reinbergs, 1982) obtained from PROC UNIVARIATE were compared to sampling standard errors using the Z score. Sampling standard errors (SE) of g_1 and g_2 were calculated by the formula (Fisher, 1950):

$$SE_{g1} = \left[\frac{6n'(n' - 1)}{(n' - 2)(n' + 1)(n' + 3)} \right]^{1/2}$$

$$SE_{g2} = \left[\frac{24n'(n' - 1)^2}{(n' - 3)(n' - 2)(n' + 3)(n' + 5)} \right]^{1/2}$$

Where n' is the sample size.

Milling energy and beta-glucan heritability were not estimated due to limited data availability.

5.3 Results

Valier and TR253 differed in SKCS hardness, milling energy, light reflectance, protein and beta-glucan (Table 5.1). Valier was harder than TR253, as measured by SKCS hardness, milling energy and light reflectance and had higher protein and beta-glucan. No differences

Table 5.1. Valier and TR253 mean grain hardness, protein, beta-glucan (2004 only) and SKCS seed weight, diameter, and moisture characteristics, 2003 and 2004.

Genotype	SKCS Hardness	Milling Energy (j)	Light Reflectance (1000 pixels/mm^2)	Protein (%)	Beta-glucan (%)	SKCS Seed		
						Wt (g)	Diam (mm)	Moist (%)
Valier	56.2 $_a$	672 $_a$	15.8 $_b$	12.1 $_a$	4.47 $_a$	50.6	2.76	9.88
TR253	51.9 $_b$	629 $_b$	18.3 $_a$	11.4 $_b$	4.01 $_b$	50.2	2.74	9.93
Mean	54.0	651	17.0	11.7	4.24	50.4	2.75	9.90
SE	0.40	5.5	0.20	0.09	0.06	0.20	0.01	0.03
Pr>t	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.06	0.05	0.11

Values in the same column followed by the same subscript do not differ (P<0.05).

were detected between Valier and TR253 for SKCS seed weight, diameter or moisture. No further analysis was performed for these three SKCS measured seed traits.

SKCS hardness, milling energy, and light reflectance differed between years (P<0.03), although ranking of Valier and TR253 did not change (data not shown). The protein level of Valier was similar across years (P>0.07) but TR253 protein was higher in 2003 compared to 2004 (P<0.03). It is not known if beta-glucan varied across years due to limited data availability (2004 only).

The population analysis of variance revealed significant differences between genotypes for SKCS hardness, milling energy, light reflectance, protein and beta-glucan (P<0.0001). Significant differences between years also existed for SKCS hardness, light reflectance and protein (P<0.0002) (data not shown). It is not known if milling energy and beta-glucan differed between years, due to limited data availability. No genotype by year interaction was detected for light reflectance or protein (P>0.05). Non-cross over genotype by year interaction was detected for SKCS hardness (P<0.0001). It is not known if genotype by year interactions affected milling energy and beta-glucan, due to limited data availability. All years/reps were combined for final analysis of grain hardness, protein and beta-glucan.

Table 5.2. SKCS hardness, milling energy, light reflectance, protein and beta-glucan midparent values and population description for the Valier/TR253 DH population, 2003 and 2004 combined.

Trait	Midparent value	Population mean	Range	Min	Max	SE	W	Pr<W
SKCS Hardness	54.0	52.9	19.0	42.5	61.5	0.21	0.993	0.34
Milling Energy (j)	651	640	158	558	716	1.70	0.996	0.71
Light Reflectance (1000 pixels/mm^2)	17.0	16.8	6.4	14.0	20.3	0.10	0.974	0.046
Protein (%)	11.7	12.0	2.7	10.8	13.5	0.03	0.993	0.27
Beta-glucan (%)	4.24	4.09	1.88	3.14	5.02	0.02	0.997	0.92

The population ranged in SKCS hardness from 42.5 to 61.5 with a mean of 52.9 (Table 5.2), milling energy from 558 to 716 joules with a mean of 640 joules, and light reflectance from 14.0 to 20.3 thousand pixels/mm^2 with a mean of 16.8 thousand pixels/mm^2. Protein varied from 10.8 to 13.5% with a mean of 12.0%, while beta-glucan varied from 3.14 to 5.02% with a mean of 4.09% (Table 5.2).

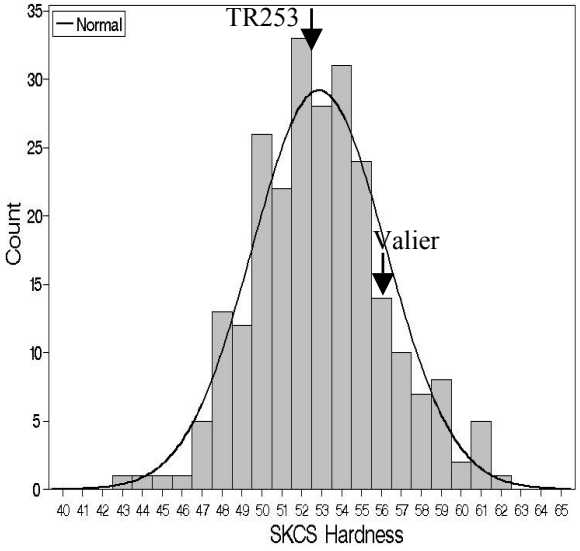

Figure 5.1. SKCS hardness distribution of Valier/TR253 double haploid population (n=245), 2003 and 2004 combined.

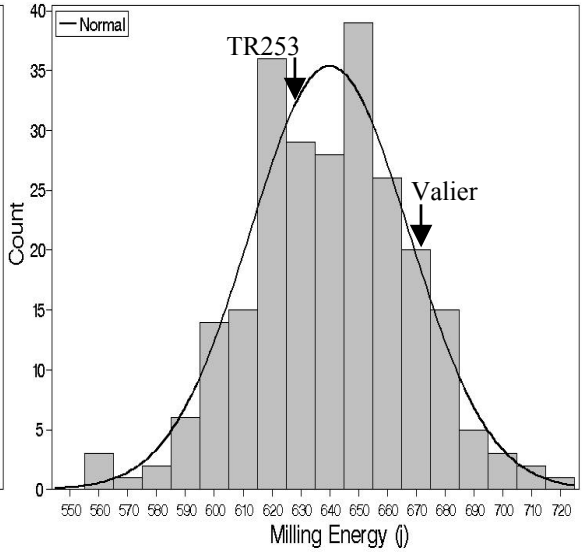

Figure 5.2. Milling energy distribution of Valier/TR253 double haploid population (n=245), 2003 and 2004 combined.

Shapiro-Wilk's (W) test for normality (Table 5.2) determined that the population was normally distributed (P>0.27) for SKCS hardness (Figure 5.1), milling energy (Figure 5.2), protein (Figure 5.3) and beta-glucan (Figure 5.4). However, light reflectance (Figure 5.5) was slightly leptokurtic and not considered normally distributed (P<0.046).

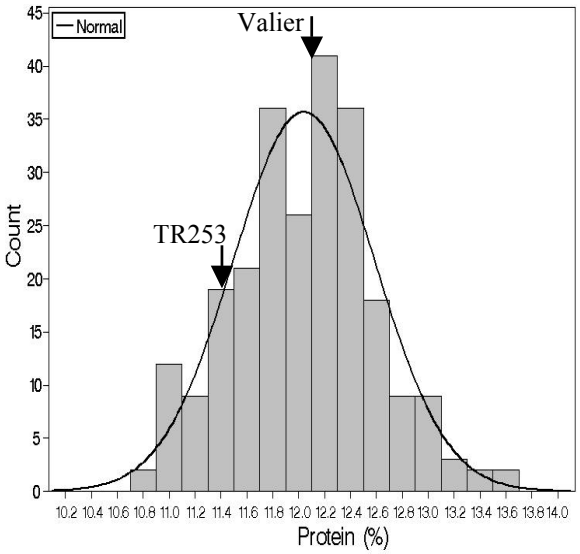

Figure 5.3. Protein distribution of Valier/TR253 double haploid population (n=245), 2003 and 2004 combined.

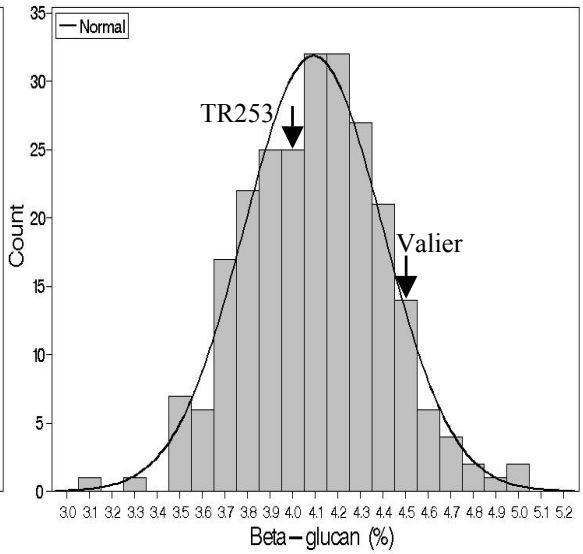

Figure 5.4. Beta-glucan distribution of Valier/TR253 double haploid population (n=245), 2004.

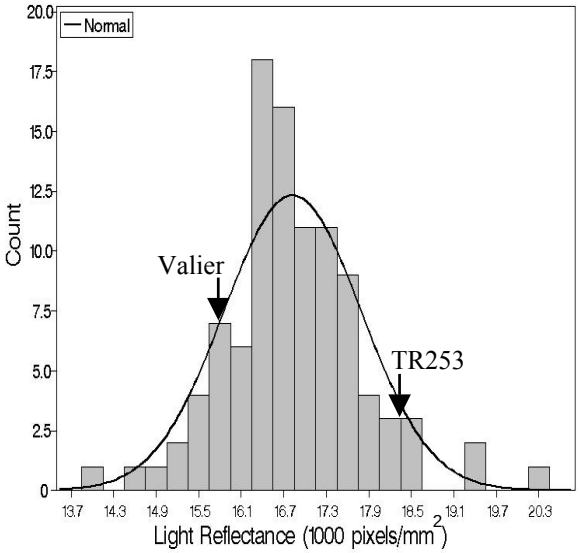

Figure 5.5. Light reflectance distribution of Valier/TR253 double haploid population (n=100), 2003 and 2004 combined.

Table 5.3. SKCS hardness, light reflectance, and protein broad-sense heritability (H^2), skewness (g_1), kurtosis (g_2) coefficients for the Valier/TR253 double haploid population.

Trait	Heritability (H^2)	Skewness (g_1)	Kurtosis (g_2)
SKCS Hardness	0.75	0.15	0.21
Light Reflectance (1000 pixels/mm^2)	0.53	0.42 *	1.77**
Protein (%)	0.41	0.06	-0.14

*Significant at 0.05 level.
**Significant at 0.01 level.

Recombination of genes in the population resulted in transgressive segregation for SKCS hardness and milling energy, with more segregants softer than TR253 (soft parent) than harder than Valier (hard parent). However, light reflectance showed few progeny harder than Valier in 2003, with none in 2004. No transgressive segregation beyond TR253 was apparent for light reflectance in either year. For protein, transgressive segregation occurred beyond the high protein parent Valier. Few segregants had less protein than TR253 in 2003 and none in 2004. No transgressive segregation for beta-glucan was apparent beyond high parent Valier, with few having less beta-glucan than TR253.

Broad-sense heritability for the Valier/TR253 DH population was estimated as 0.75 for SKCS hardness, 0.53 for light reflectance and 0.41 for protein (Table 5.3). Skewness (g_1) and kurtosis (g_2) coefficients were not significantly different than zero for SKCS hardness ($P>0.17$) and protein ($P>0.33$), indicating an absence of epistasis (Table 5.3). However, a positive g_1 ($P<0.04$) for light reflectance suggested complementary gene interaction affected light reflectance (Table 5.3). This result was further substantiated with g_2 also being significantly greater than zero for light reflectance ($P<0.0002$). Therefore, narrow-sense heritability equals broad-sense heritability for SKCS hardness and protein, but not light reflectance.

Table 5.4. Valier/TR253 double haploid population Pearson correlations between SKCS hardness, milling energy, light reflectance, protein, and beta-glucan.

	SKCS Hardness	Milling Energy (j)	Light Reflectance (1000 pixels/mm^2)	Protein (%)	Beta-glucan (%)
SKCS Hardness	1				
Milling Energy (j)	0.44	1			
	P<0.0001				
Light Reflectance (1000 pixels/mm^2)	-0.28	-0.46	1		
	P<0.005	*P<0.0001*			
Protein (%)	-0.17	0.18	-0.14		
	P<0.008	*P<0.004*	*P=0.15*	1	
Beta-glucan (%)	0.42	0.43	-0.18	0.22	
	P<0.0001	*P<0.0001*	*P=0.08*	*P<0.0006*	1

N=245 for all analysis except light reflectance (N=100).

Pearson's correlation analysis is summarized in Table 5.4. SKCS hardness (n=245) was positively related to milling energy (R^2 = 0.20, P<0.0001) (Figure 5.6), and beta-glucan (R^2 = 0.18, P<0.0001) (Figure 5.7). A minimal negative correlation was detected between SKCS hardness and both light reflectance (r = -0.28, P<0.005, n=100) and protein (r = -0.17, P<0.008, n=245). Milling energy was negatively related to light reflectance (R^2= 0.21, P<0.0001, n=100)

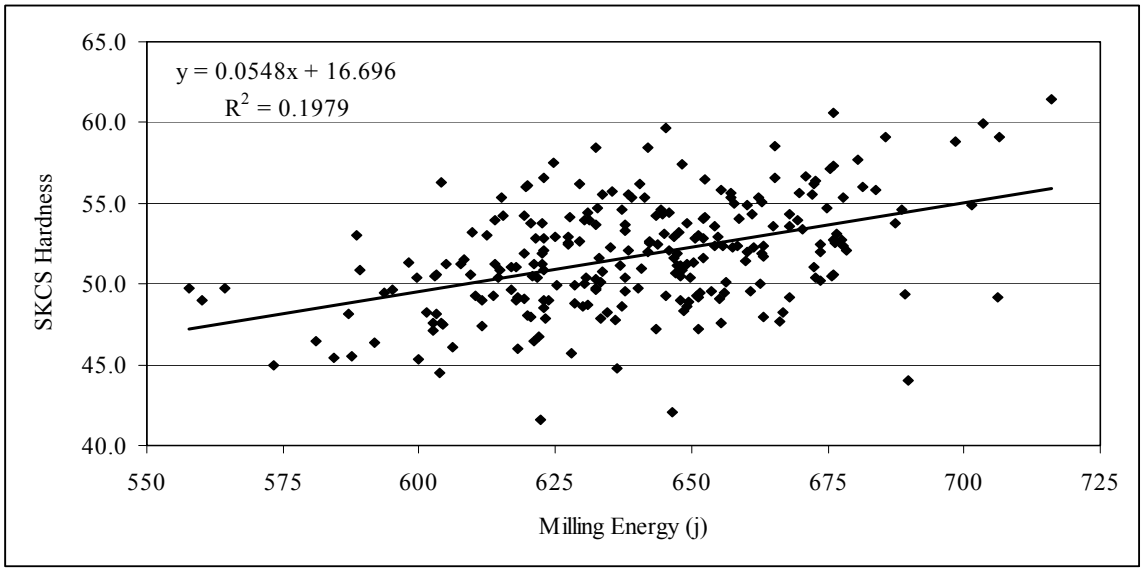

Figure 5.6. Relationship between SKCS hardness and milling energy for 245 Valier/TR253 double haploid genotypes.

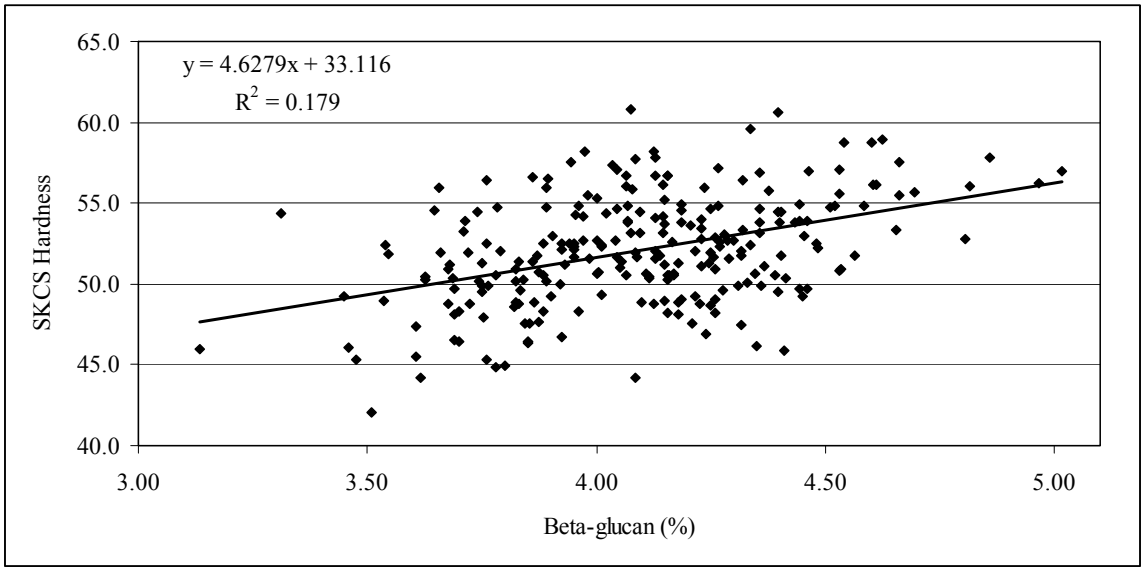

Figure 5.7. Relationship between SKCS hardness and beta-glucan for 245 Valier/TR253 double haploid genotypes.

(Figure 5.8) and positively related to beta-glucan (R^2 = 0.19, P<0.0001, n=245) (Figure 5.9). A minimal positive correlation was detected for milling energy and protein (r = 0.18, P<0.004, n=245). Light reflectance showed no correlation with either protein or beta-glucan (P>0.08, n=100).

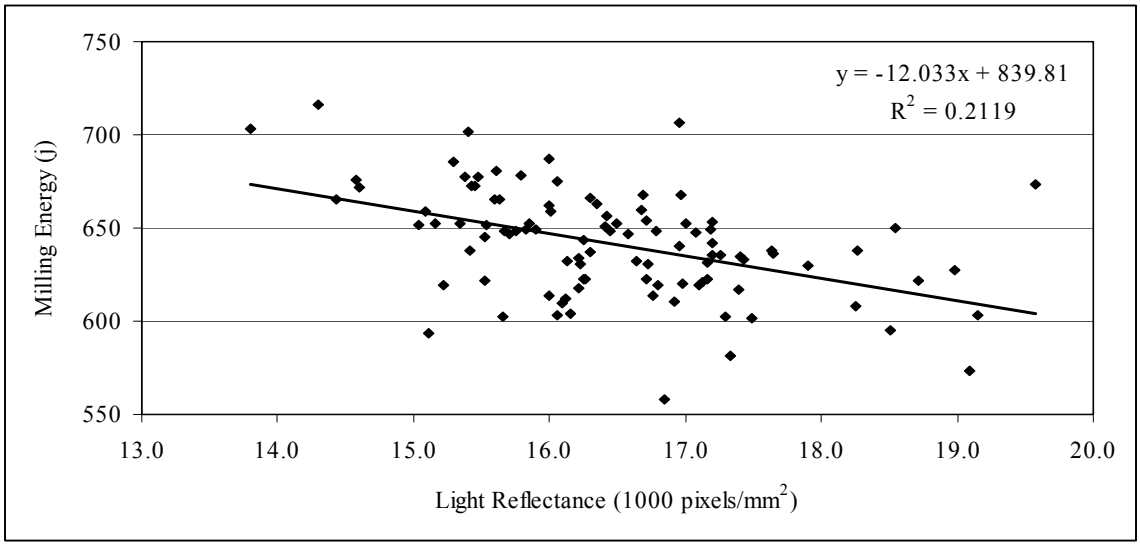

Figure 5.8. Relationship between milling energy and light reflectance for 100 Valier/TR253 double haploid genotypes.

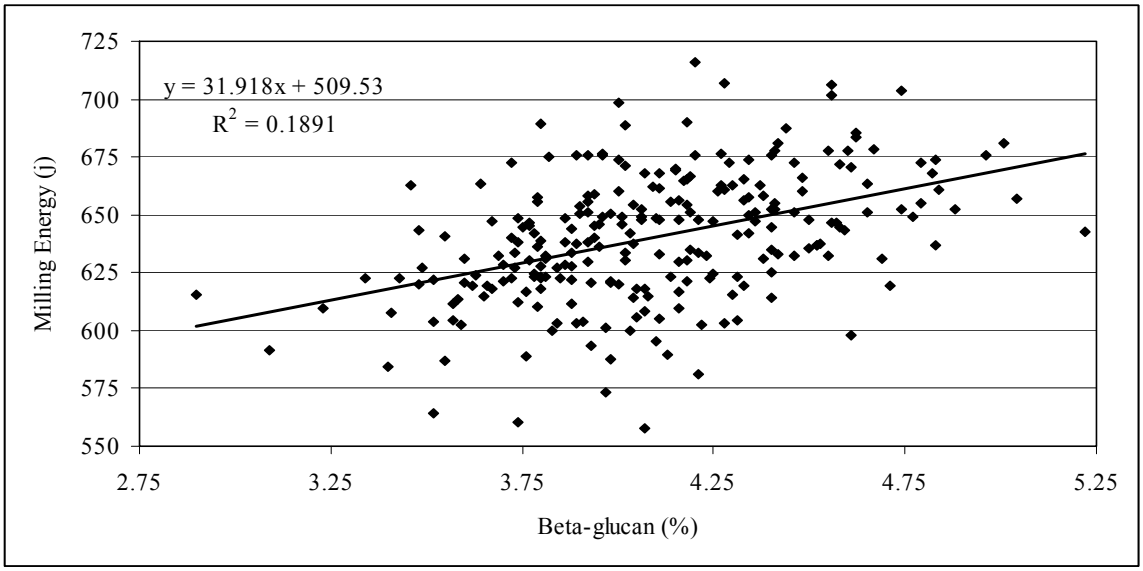

Figure 5.9. Relationship between milling energy and beta-glucan for 245 Valier/TR253 double haploid genotypes.

5.4 Discussion

Similar to results reported in the genotype experiment (Chapter 4), Valier had significantly harder grain than TR253 and significantly more protein and beta-glucan. The DH population was normally distributed for SKCS hardness, milling energy, protein and beta-glucan, indicating quantitative inheritance (Poehlman and Sleper, 1995). The unexpected absence of normality for light reflectance may be attributed to the smaller sampling of the population, where only 100 progeny were tested rather than 245 as with SKCS hardness and milling energy. Furthermore, the methodology may have been restricted in distinguishing smaller variations between progeny. Examination of the skewness/kurtosis would however suggest that light reflectance was influenced by complementary gene interaction.

Transgressive segregation was detected for SKCS hardness, milling energy, and protein, with minor amounts for beta-glucan and light reflectance. SKCS hardness, milling energy, and protein showed bi-directional transgressive segregation, suggesting that parents contributed both positive and negative alleles for these traits. No transgressive segregation was detected beyond the high beta-glucan Valier parent or the soft TR253 for light reflectance. Morris et al. (1999) and Thomas et al. (1996) similarly reported transgressive segregation in both directions, for SKCS hardness in wheat and milling energy in barley, respectively. In the current study, more softer segregants were detected than hard for SKCS hardness and milling energy, suggesting more dominant gene action for softness. In contrast to hardness, more high protein segregants were detected in the population than soft. Thomas et al. (1996) also reported transgressive segregation beyond both parents for barley protein.

The SKCS hardness heritability of 0.75 was lower than the previous published range of 0.82 to 0.96 reported by Fox et al. (2007b). In the current study, the SKCS hardness population range of 42 to 62 for TR253/Valier was considerably narrower than the range of 35 to 89 reported by Fox et al. (2007b) for 40 diverse breeding lines and commercial varieties. The narrower range may have hindered proper determination of genetic variance, resulting in a lower estimate of heritability. However, the difference may also be related to the different techniques used to measure heritability. In the current study, 245 DH progeny from the same genetic background were evaluated for hardness, compared to 40 breeding lines selected from a diverse genetic background in the Fox study. In the current study, broad and narrow-sense heritability were equal for SKCS hardness, due to the absence of epistasis in this DH population. In

contrast, Thomas et al. (1996) did report epistasis influencing another hardness measurement, milling energy.

Light reflectance heritability of 0.53 was lower than that for SKCS hardness, suggesting population variability was influenced similarly by environmental and genetic influences. As previously noted, the methodology may have been restricted in distinguishing smaller variations between progeny, resulting in more experimental variance and a lower heritability estimate. In the current study, heritability was reported only as broad-sense for light reflectance, due to detection of complementary gene interaction. As mentioned previously, these results are similar to epistasis detected by Thomas et al. (1996) with milling energy.

Low protein heritability of 0.41 suggested more environment than genetic influence on population variability. However, the result was within the range of heritability reported by Thomas et al. (1996) of 0.18 to 0.70. Similar to SKCS hardness, no epistasis was detected; therefore broad-sense equaled narrow-sense heritability. Thomas et al. (1996) also reported no epistasis for protein in a DH population.

Beta-glucan heritability was not estimated in the current study due to limited data availability. However, research by Lance (1984) reported narrow-sense heritability for barley beta-glucan of 0.66, 0.69 and 0.75 for F_2, F_3 and F_4 generations, respectively.

Correlations between traits across this population showed some similarities to those for the varieties reported in the genotype experiment (Chapter 4). SKCS hardness was positively correlated with milling energy while milling energy was negatively correlated with light reflectance and positively with beta-glucan. Light reflectance again showed no association with beta-glucan. However, unlike the genotype experiment, SKCS hardness showed little association with protein but was positively correlated with beta-glucan. Milling energy and light reflectance were not associated with protein.

The positive relationship between SKCS hardness and beta-glucan is similar to that reported by Washington et al. (2001) and Fox et al. (2007b). However, Fox et al. (2007b) reported separate correlations for four site years and indicated that the correlation was strongly affected by environmental influences. In the current study, it is not known if environment affected the SKCS hardness-beta-glucan correlation due to the limited beta-glucan data availability (2004 only). However, the genotype experiment beta-glucan results (Chapter 4) indicated non-cross over genotype by environment interaction.

In the current study, SKCS hardness showed no correlation with grain protein, similar to reports by Washington et al. (2001). However, this is in contrast with Fox et al. (2007b), who reported a significant negative relationship (r = -0.61 to -0.99 range) for three of four site years. The current study found no genotype by environment interaction for protein and only non cross-over interaction for SKCS hardness. Correlation analysis of each year's SKCS hardness and protein resulted in r = -0.24 and 0.04 for 2003 and 2004, respectively.

The current study further substantiates the relationship between milling energy and beta-glucan as reported in the genotype experiment and reported by Henry and Cowe (1990). However, the milling energy-protein correlation reported in the genotype experiment was minimal in this DH population and in contrast to findings by Allison et al. (1979a).

The current study indicates that the Valier/TR253 DH population was normally distributed for SKCS hardness, milling energy, protein and beta-glucan, indicating quantitative inheritance for these traits. However, light reflectance was not normally distributed, with significant kurtosis suggesting complementary gene interactions. Transgressive segregation was detected for SKCS hardness and milling energy beyond both parents. However, fewer hard segregants may increase the difficulty of trait selection by requiring larger population sizes. Nonetheless, heritability was high for SKCS hardness, making selection for this trait feasible for breeding.

6. GENERAL DISCUSSION

The development of slower degrading barley for ruminants should assist in alleviating health problems associated with rapid starch degradation in the rumen. Once identified, breeding and selection for quality corresponding with the slow DMDR trait should be possible. Unfortunately, the current research demonstrated inconsistency for DMDR, with genotype DMDR significantly and differentially affected by growing environment.

In this study, DMDR genotype by environment interaction was non-crossover in nature, with genotype Valier either degrading more slowly (as expected) or being no different than TR253. While SEM micrographs of Valier grain indicated more continuous starch-protein matrices from environments demonstrating slower DMDR, similar continuous starch-protein matrices were also found for TR253 from environments showing no differential DMDR. This demonstrated that SDMD genotypes may behave "normally" and "normal" types may behave as SDMD types, depending on the growth environment. However, other research by Lehman et al. (1995) reported no genotype by environment interaction for eight barley genotypes. Therefore, it may remain feasible to develop varieties with consistent slow DMD performance. Unfortunately for the current study, Valier was a poor choice as a test subject and donor parent due to its inconsistent SDMD behavior.

For DMDR selection to be effective, a better understanding of the environmental influences on DMDR, such as growing season temperature and precipitation, is required. This would help direct evaluation to certain growing locations that would provide ideal selection pressure. However, multiple site evaluation of DMDR must be completed to verify consistent performance before variety release to ensure maximum benefit for the end-user.

Reliable and effective DMDR measurement can be problematic. Measuring DMDR using *in situ* degradation kinetics, as in the current study, is limited in numerous ways. First, the number of treatments tested in one experiment is small due to limited rumen capacity and the large number of samples required for incubation time-periods. The current evaluation of eight treatments (2 genotypes x 4 environments) measured at six time-periods (0-hour to 24-hour) was

near rumen capacity and time limits. Because the rumen is a living, volatile environment, extending completion of an experiment to increase the treatment number introduces more animal/rumen variability, lessening accuracy of the results. Including a standard sample to gauge rumen variability during each multiple run could help determine the significance of *in situ* results. This could be a carbohydrate-protein-cellulose-based substance with a consistent chemical structure, fabricated for such use.

Second, *in situ* degradation measurement is time consuming, again restricted by the number of possible treatments in the rumen and the requirement of a minimum 24-hour degradation time-period. Modified, quick methods to measure degradation have been utilized by others. For example, Bowman et al. (2001) measured extent of DMD after only 3 hours of rumen degradation. While providing an idea of the potential DMDR, this is only a glimpse of the actual DMDR. Quite possibly, some genotypes may have initially delayed DMD but rapid DMDR once degradation is initiated. In the current study, no differences were detected between Valier and TR253 in extent of rumen degradation after two or four hour incubation from any growing location (data not shown). As such, had only that period been evaluated, it would have been mistakenly concluded that there were neither differences between Valier and TR253, nor any genotype by location interaction.

Third, *in situ* experiments are expensive to perform, compared to standard physical and chemical techniques, such as bulk density, kernel weight, protein, beta-glucan or grain hardness determination. Therefore, *in situ* evaluation remains impractical from a plant breeding program perspective where screening large numbers of putative slow DMDR lines is necessary.

Grain hardness was not clearly related to DMDR in this study, as measured by SKCS hardness, SEM, or particle size. Valier and TR253 had similar sized particles and yet samples differed significantly for DMDR from certain locations. Valier was harder at all sites. However, since only two genotypes were examined *in situ*, inferences that can be made from this study are limited. Another trial with three or more distinctive genotypes differing in hardness/particle size could establish a more defined relationship between SKCS hardness, milling energy, particle size and DMDR.

Although the current study did not confirm the relationship of particle size to DMDR, research by Surber et al. (2000) indicated that larger mean particle size with reduced ADF could be used as indirect criteria to select for reduced rumen DM degradation. Feed particle size

analysis may remain a reasonable basis for selection until more studies are completed. The current study found that mean particle size was related to beta-glucan and to a lesser extent protein, as these components likely help maintain cohesion of endosperm cells and starch granules during processing. Selecting barley with high beta-glucan and protein should result in larger particle size and potentially slower DMDR. This theory is further substantiated by the research of Zheng et al. (2000), who found that low beta-glucan hulless barley genotypes (4%) had concentrated beta-glucan levels in the subaleurone and adjacent endosperm area rather than throughout the grain. In contrast, high beta-glucan genotypes (7 to 9%) had uniformly distributed beta-glucan throughout the endosperm, with the starchy endosperm containing more than the subaleurone (Zheng et al., 2000). Therefore, high levels of beta-glucan dispersed throughout the endosperm would potentially lessen the impact of processing and result in larger particles. More research is also required to determine if specific proteins may influence particle size in barley, as is the case for wheat.

The effect of high beta-glucan barley in ruminant diets is not well understood. Engstrom et al. (1992) reported that barley beta-glucan from 3.5 to 4.8% caused no problems for rumen degradation. In the current study, the beta-glucan levels of both Valier (4.35%) and TR253 (4.01%) would be considered normal. It is of interest that Foley et al. (2006) found that waxy barley had a slower rate of starch degradation than normal barley, unrelated to degree of processing or feed particle size, when halved grains were tested *in situ*. They suggested that greater soluble fibre (i.e. beta-glucan) in the waxy type may have decreased degradation rate compared to that for the normal starch genotype. Although beta-glucan levels were not reported in the Foley research, Zheng et al. (2000) found that waxy and high amylose hulless barley contained more beta-glucan (7 to 9%) than normal starch genotypes (4%).

Breeding programs require rapid, efficient, inexpensive, and effective screening techniques. Therefore, not all hardness methods tested in the current study would be suitable. Mean feed particle size measurement was cumbersome, with two separate steps (rolling and sieving) requiring a total of 10 to 15 minutes per sample. The system is inexpensive, with only the cost of the feed roller and sieve shaker, which are readily purchasable. However, it requires a larger quantity of grain (50 grams used in the current study) and therefore cannot be used for early generation screening. Based on research by Surber et al. (2000), particle size analysis is

effective in selecting for reduced extent of degradation (after 3 hours) and may be useful for later generation selection.

Milling energy is quick and efficient, requiring only one minute and five grams of seed per sample. The current study found that milling energy was closely related to mean particle size and was influenced by beta-glucan and protein. Although the current study was unable to determine heritability of milling energy, Thomas et al. (1996) reported it high. Milling energy would be a good screening tool that may help indirectly select for slower DMDR. However, the "Compara-mill", used to determine milling energy, is no longer being manufactured. Therefore, other institutions are unable to purchase the equipment for use in selection.

SKCS hardness evaluation is rapid (three minutes per sample), efficient, and inexpensive (after initial purchase cost) and easily obtainable. The SKCS unit requires 300 seed per evaluation, although fewer could be analyzed depending on screening stringency. Although highly heritable, current study results did not demonstrate a strong enough relationship to select effectively for particle size or DMDR. The crushing action of the SKCS may give differing results than the shearing action of the mill and roller mill used in the milling energy and feed particle size analysis systems.

Light reflectance did not effectively select for particle size or DMDR. This method was more time-consuming than any other hardness test, with a combined time of about 45 to 60 minutes per sample. Although equipment was easily purchasable, labour costs would be higher than for other hardness tests.

Scanning Electron Microscopy is a very detailed observation of grain hardness and provides only a partial view of the whole endosperm. It is also time-consuming, with sectioning, coating, and examination of individual samples requiring a minimum of 20 minutes per sample. Multiple seeds, sections and micrographs are required to collect this detailed information, making it cost-prohibitive. Therefore, SEM is impractical for screening purposes.

Beta-glucan and protein analyses are relatively rapid, efficient and inexpensive routine laboratory procedures. In the current study, beta-glucan and protein were associated with mean feed particle size and milling energy. The current study was unable to determine beta-glucan heritability but determined protein heritability was low. However, Lance (1984) reported high beta-glucan heritability. These two procedures, especially beta-glucan analysis, may be the most effective screening tool for larger feed particle size and thus indirectly, slow DMDR.

In the current study, genotype DMDR was inconsistent across environments, thereby contributing to the difficulty of accurately selecting slower DMDR genotypes in a breeding program. Large-scale DMDR screening would be costly, laborious, and impractical. Conversely, barley grain hardness is highly heritable, relatively inexpensive and efficient. Unfortunately, neither SKCS hardness, SEM, nor feed particle size analyses clearly differentiated DMDR between genotypes. Breeding for high beta-glucan and protein may be good initial selection criteria for indirect selection. More research is required to elucidate fully the relationship between grain hardness, beta-glucan, protein, and DMDR in barley.

7. CONCLUSIONS

1. Valier had a slower rate of dry matter disappearance (Kd) than TR253 at Brandon and Saskatoon (SF) but was similar to TR253 at Saskatoon (KCRF) and Wakaw.

2. Higher SKCS hardness in Valier compared to TR253 across sites did not result in consistent slower rates of dry matter disappearance for Valier.

3. SEM observations of the endosperm starch-protein matrices did not account for the differences in rate of dry matter disappearance between Valier and TR253, grown at multiple sites.

4. Hardness measurement methodology (SKCS hardness, milling energy, light reflectance) ranked genotypes similarly across environments, although levels varied at each.

5. McLeod and CDC Bold were consistently the hardest and softest genotypes, respectively, of all genotypes tested with SKCS hardness, milling energy, light reflectance and mean feed particle size.

6. Milling energy was consistently related to feed particle size.

7. Milling energy appeared to be influenced by protein and beta-glucan, with higher levels imparting harder grain.

8. SKCS hardness and light reflectance were not correlated with protein or beta-glucan.

9. Feed particle size may be influenced by protein and beta-glucan content.

10. SKCS hardness, milling energy, protein and beta-glucan show normal distributions and are quantitatively inherited.

11. Light reflectance was not normally distributed and may be affected by epistasis.

12. Broad-sense heritability for SKCS hardness was high, while for light reflectance and protein, it was lower.

13. Breeding for high beta-glucan and protein genotypes may be reasonable initial criteria for indirect selection for slow DMDR.

8. SUGGESTIONS FOR FUTURE RESEARCH

1. *In situ* dry matter disappearance testing of barley isolines, widely differing in SKCS hardness, milling energy and/or light reflectance, grown in multiple environments.

2. *In situ* dry matter disappearance testing of barley isolines, widely differing in beta-glucan content, grown in multiple environments.

3. *In situ* dry matter disappearance testing of barley isolines, widely differing in mean feed particle size, grown in multiple environments.

4. Identifying endosperm protein/s responsible for barley grain hardness.

5. Examining the relationship between arabinoxylans, beta-glucan, SKCS hardness, milling energy and mean feed particle size.

6. Examining the effects of hull characteristics on SKCS hardness, milling energy and mean feed particle size.

7. Examining the inheritance and heritability of milling energy, mean feed particle size, and beta-glucan.

8. Identifying QTLs for SKCS hardness, milling energy, mean feed particle size, and beta-glucan.

9. LITERATURE CITED

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